
Cover Letter

May 19, 2004

DEP/DPH Advisory Committee on Health Effects;
Invited scientists; and,
Interested Parties

Dear Sir or Madam:

The purpose of this letter is to briefly discuss the status of the Massachusetts Department of Environmental Protection's (MA DEP's) draft *Perchlorate, Toxicological Profile and Health Assessment (May, 2004)*, which has been recently posted on MA DEP's web page. This draft document has been prepared by toxicologists and risk assessors of MA DEP's Office of Research and Standards (ORS), with input from the DEP/DPH Advisory Committee on Health Effects and invited scientists. It provides a review of the scientific information on the toxicity of perchlorate and presents the derivation of a proposed reference dose, which is an estimated dose to which a person can be exposed without an appreciably adverse health risk. MA DEP's proposed reference dose was derived using a weight of the evidence approach, where the entire body of the available data on perchlorate toxicity was critically evaluated for consistency and biological plausibility in order to derive a reference dose that is appropriately protective for all members of the population.

The reference dose is the starting point for establishing regulatory standards for perchlorate and provides the scientific basis for MA DEP's interim guideline for perchlorate in Massachusetts' drinking water. MA DEP is planning to adopt cleanup standards for hazardous waste sites under the Massachusetts Contingency Plan (MCP, MGL Chapter 21E) and, potentially, for a drinking water standard, pending findings on public drinking water supply testing.

MA DEP started its work to collect data on perchlorate and to set standards prior to the decision for the U.S. Environmental Protection Agency's draft perchlorate toxicological assessments to be reviewed by the National Academy of Sciences. MA DEP will take the results of this review into account in its standard setting processes if it is ready and on schedule (September, 2004).

When MA DEP issues proposed standards, there will be a public hearing and comment period where we will accept comments on the draft toxicity assessment, proposed reference dose and standards.

Very truly yours,

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Director, Office of Research and Standards

Final Draft

Perchlorate

Toxicological Profile And Health Assessment

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May 2004

Advisory Committee

DEP/DPH Advisory Committee on Health Effects

DEP is very appreciative of the participation of the members of the Health Effects Advisory Committee in the scientific peer review of DEP's draft toxicity assessment. Their generous commitment of time and tremendous expertise have been extremely helpful to our efforts on this important issue. The participation of independent public health scientists is a critical component of our state's efforts to protect public health and the environment in MA.

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Acknowledgement

DEP wishes to acknowledge representatives of the U.S. Department of Defense for sharing information on the toxicity and exposure of Perchlorate with DEP and the Advisory Committee. The U.S. Department of Defense representatives include:

Mick Major, Ph.D., U.S Army Center for Health Promotion and Preventive Medicine;
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EXECUTIVE SUMMARY

Background

Ammonium perchlorate is widely used as a component of propellants for rockets, missiles, and fireworks. It was discovered in surface and ground waters in the United States in the late 1990s, typically around military operations, defense contracting or manufacturing facilities (U.S.EPA, 2000). Perchlorate has been found at levels up to 500 ppb in groundwater at the Massachusetts Military Reservation (MMR) on Cape Cod. It was also detected in public and private drinking water supplies in the adjacent town of Bourne at concentrations around 1 ppb. In March 2002, faced with contamination problems and no federal or state drinking water standard for perchlorate, the Bourne Water District (BWD) requested guidance from the Massachusetts Department of Environmental Protection (MA DEP) on perchlorate in drinking water. To respond in a timely way, DEP's Office of Research and Standards (ORS) reviewed available information on the toxicity of perchlorate, including the draft U.S. EPA health assessment for perchlorate (U.S.EPA, 2002), which contains a draft reference dose and an associated drinking water limit of 1 ppb for perchlorate. Using this most current scientific information, in April 2002 MA DEP provided the BWD with interim advice recommending that sensitive subgroups, namely pregnant women, infants, children up to the age of 12 and individuals with hypothyroidism should not drink the water when perchlorate concentrations exceed 1 ppb. The Massachusetts Department of Public Health (MA DPH) supported this interim advice and EPA Region 1 issued a statement indicating that the advice was health protective. Due to an immediate need for standards to address perchlorate at hazardous waste sites and in drinking water in this area of Massachusetts, MA DEP made a decision in January 2003 to initiate the process of adopting a MA reference dose (RfD) for perchlorate.

It has been well established that perchlorate interferes with iodide transport into the thyroid gland at the sodium (Na)-iodide-symporter (NIS). Perchlorate also promotes the discharge of accumulated iodide from the thyroid gland and interferes with other cellular processes involved in thyroid function (e.g., the pendren protein). These effects can lead to decreased synthesis of the thyroid hormones thyroxine (T4) and triiodothyronine (T3), and increased release of the pituitary hormone, thyroid-stimulating hormone (TSH). Due to these effects, perchlorate has historically been used to treat patients with Graves' disease, a disorder characterized by hyperthyroidism. Perchlorate treatments are able to inhibit the excessive synthesis and secretion of thyroid hormones by decreasing the uptake of iodide into the thyroid gland and by promoting the discharge of accumulated iodide in the thyroid in these patients.

Studies in iodine-deficient populations are relevant to understanding perchlorate's toxicity, since the mode of action of perchlorate is primarily to produce an iodine-deficient thyroid. Iodine deficiency in pregnant mothers results in an insufficient supply of thyroid hormones to the fetus. In areas where iodine intake is marginal (<100 ug/day), low serum T4 and T3 levels (hypothyroidism), enlarged thyroid and goiter were often detected in pregnant women (Crooks et al., 1967; Glinioer et al., 1990; 1992; 1995; Smyth et al. 1997; Caron et al., 1997; Brent, 1999; Kung et al., 2000). The maternal hypothyroidism caused impaired intellectual and physical development in offspring. Children born to iodine-deficient mothers have a loss of 5-13 intelligent quotient (IQ) points compared to children born to iodine-sufficient mothers

(Bleichrodt et al. 1989; Vermiglio, et al., 1990; Fenzi et al., 1990; Vitti et al., 1992; Aghini-Lombardi et al., 1995; Tiwari et al., 1996; Azizi et al., 1993; Shresta, 1994; Bleichrodt and Born, 1994; Van den Briel et al, 2000). Even in iodine sufficient areas, children of women with free T4 levels below the 5th and 10th percentile at 12 weeks of gestation had significantly lower scores on psychomotor development at 10 months of age compared to children of mothers with higher T4. Severe iodine deficiency (<20 ug/day iodine intake) has been shown to cause stillbirths, congenital anomalies, increased perinatal mortality, and cretinism. Cretinism is characterized by deaf mutism, mental and growth retardation, goiter and various neurologic disorders (Hertzel, 1983; Laurberg et al., 1998; Stanbury et al., 1998; Laurberg, 2000).

Taken together, these studies indicate that the most sensitive subgroups for iodine deficiency are:

- (a) pregnant women, especially marginally iodine-deficient or iodine-deficient pregnant women;
- (b) fetuses and infants of these women;
- (c) children; and
- (d) hypothyroid individuals.

To see if perchlorate could cause effects similar to those associated with iodine deficiency in the human population, in 1997 the U.S. Air Force, U.S. EPA, the Perchlorate Study Group (PSG), TERA (i.e., Toxicology Excellence for Risk Assessment), and others, developed a research program and protocols for studies on perchlorate to fill data gaps. The U.S. Department of Defense (DOD) funded these studies. The studies on perchlorate demonstrated the following effects associated with perchlorate exposure, which are consistent with its proposed mode of action:

- inhibition of iodide uptake in both humans (Lawrence et al., 2001; and Greer et al., 2002) and animals (Yu et al., 2000);
- decrease of serum T4 and T3 levels and increase of TSH levels (Caldwell et al., 1995; Springborn Laboratories Inc, 1998; Argus Research Laboratories, Inc., 1998a, 1999, 2001);
- thyroid hypertrophy (increased cell size), hyperplasia (increased cell number) across life-stages, and tumors in F1 generation rats (rats exposed *in utero* and throughout lactation)¹ (Caldwell, 1995; Argus Research Laboratories, Inc., 1998a, 1999);
- alteration in brain morphometry (form and structure) and behavior in rat pups that were exposed *in utero* and after birth (Argus Research Laboratories, Inc., 1998a, 2001; Bekkedal et al., 2000); and,
- enhancement of contact dermatitis induced by the known dermal sensitizer 2,4-dinitrochlorobenzene (DNCB) (Burleson Research Technologies (2000).

¹ Perchlorate was found to be nongenotoxic in various *in vitro* and *in vivo* studies, suggesting that the mechanism of tumor formation might be perturbation of the thyroid and pituitary hormone homeostasis (ManTech Environment Technology, 1998; Zeiger, 1998).

Toxicity Assessment and Reference Dose Derivation

ORS has reviewed human and animal toxicity studies in preparing its toxicity assessment of perchlorate. The Office has also sponsored three meetings of its Advisory Committee on Health Effects, an external scientific peer review group, along with scientific representatives of the U.S. Department of Defense on the toxicity of perchlorate. Information and comments received from these meetings were taken into account in the assessment and derivation of a MA RfD. In establishing the RfD, ORS has taken a weight of evidence approach where the available data on perchlorate toxicity was critically evaluated for consistency and biological plausibility in order to select an appropriate point of departure to derive an RfD for this compound that is appropriately health protective for all of the members of the population. ORS notes that the concordance of effect levels observed for several endpoints, including iodide uptake inhibition, hormone effects and brain morphometry, as well as the consistency in observed effects with those that would be expected based on the perchlorate proposed mode of action provides a strong basis for establishing an RfD. ORS' assessment has included consideration of both the human and animal data to ensure that perchlorate's impacts on various life stages is taken into account. Whereas the human data represent perchlorate's effects on healthy adults, the animal data address the effect of perchlorate on the fetus and neonates who have limited thyroid hormone synthesis capacity.

The target tissue for perchlorate toxicity is the thyroid gland. The initial step leading to altered pituitary and thyroid hormone levels, brain morphometry, motor activity, thyroid structure, and tumor production is interference with iodide uptake and metabolism at the thyroid primarily attributable to inhibition of iodide uptake at the NIS by perchlorate. There is also potential interference with other components (e.g., the pendren protein) of thyroid function. The observed effects of perchlorate on the thyroid and the brain are consistent with its proposed mode of action. Changes in thyroid weight, thyroid and pituitary hormone levels, and thyroid histopathology (colloid depletion, hypertrophy, and hyperplasia) were consistently altered across an array of experimental studies. Changes in thyroid hormone levels (T3, T4) during gestation and development and changes in brain morphometry and behavior were observed in rat pups exposed *in utero* and postnatally.

ORS has selected two key studies for the RfD derivation: the neurodevelopmental study conducted by Argus Laboratories (2001); and, the human oral intake study conducted by Greer (Greer et al. 2002).

In the neurodevelopmental study (Argus Laboratories Inc. 2001), perchlorate treatment altered thyroid and pituitary hormone levels and brain morphometry in young animals exposed *in utero* and postnatally. This study was conducted to confirm brain morphometry alterations observed in an earlier study (Argus Laboratories Inc. 1998). In several studies including the Argus Laboratories Inc., 2001 study, 0.01 mg/kg-day is the Lowest Observed Adverse Effect Level (LOAEL) identified for thyroid and pituitary hormone alterations and for changes in brain morphometry (Table 1). A LOAEL value of 0.01 mg/kg-day for ammonium perchlorate (0.0085 mg/kg-day perchlorate anion) was selected as a basis for the RfD derivation using animal data (Table 1).

In the Greer study (Greer et al., 2002), groups of healthy iodine-sufficient adult subjects were treated with oral perchlorate doses ranging from 0.007 to 0.5 mg/kg-day for 14 days. A minimally effective LOAEL identified in this study based on iodide uptake inhibition was 0.007 mg/kg-day (Table 1). ORS does not consider this dose as a NOAEL due to the small number of individuals and associated low power in this dose group to detect a statistically significant effect.

Table 1*. Summary of Endpoints and Associated Lowest Values of LOAELs and BMDLs Used in Weight of Evidence Approach to Deriving an RfD for Perchlorate

Species	Endpoints Evaluated at Different Life-Stages	LOAEL (mg/kg-day)	BMDL (mg/kg-day)	References
Rat	T4 Levels	0.01	** 2.86×10^{-7} (PND21) **0.004 (GD21 dam)	Argus, 2001; Springborn, 1998
	T3 Levels	0.01		Argus, 2001; Springborn, 1998
	TSH Levels	0.01		Argus, 2001; Springborn, 1998
	Thyroid Colloid Depletion		0.009	Argus, 1998a
	Thyroid Hypertrophy		0.008	Springborn, 1998
	Thyroid Hyperplasia		0.0004 (P2 Generation)	Argus, 1999
	Brain Morphometry Changes (corpus callosum, striatum, cerebellum)	0.01 (PND21 pups)		Argus, 2001
Human	Radioiodide Uptake Inhibition	0.007	0.002 mg/kg-day (U.S.EPA, 2003)	Greer et al., 2002

* Summarized from Tables 10 and 11

** Postnatal day 21 pups and gestation day 21 dams (Argus, 2001)

For endpoints analyzed using the benchmark dose (BMD) model, the lower limits of the 95 percent confidence intervals on the benchmark doses (BMDLs) also support a LOAEL of 0.01 mg/kg-day. As shown in Table 1, some of the BMDLs, however, especially those for hormonal alterations, are much lower than the selected LOAEL. These BMDL values were considered by ORS but not selected as a basis for calculating a toxicity value for perchlorate, because of their great variability and questions about applicability of the BMD approach to the data set.

To establish the RfD, ORS has applied standard U.S. EPA (2002b) uncertainty factors to the animal and human LOAELs. Based on ORS' judgment and consideration of discussions at Advisory Committee meetings, several sets of justifiable uncertainty factors were identified and applied to the animal and human LOAELs to arrive at a number of RfDs which span a range of possible true values (Figure 1). This range was used to identify an RfD based on the convergence of all of the data.

As shown in Figure 1, the resulting RfD range derived using the animal and human data overlap between 2.3×10^{-5} and 3×10^{-5} mg/kg-day. The RfD value at the higher end of this range is only 1.3 times the value in the lower end of the range. Since MA DEP as a rule develops a single RfD and not a range, the value of 3×10^{-5} mg/kg-day was selected as the point estimate for the RfD. This value is in the range of overlap of values derived using the human and animal data and was selected because it is based on the more robust animal database where studies were conducted using the most sensitive life stages and endpoints. For comparison, the BMDL estimated by the U.S. EPA (2003) using the Greer et al. (2002) study is included in Table 1. U.S.EPA (2003) considered the BMDL as a NOAEL and applied a total uncertainty factor of 100 (10 for human variability and 10 for database deficiency and derived an RfD of 2×10^{-5} mg/kg-day for perchlorate. This value is equivalent to the RfD derived by MA DEP using the same data and the LOAEL/NOAEL approach.

In conclusion, based on the weight of the evidence, this report proposes an RfD of 3×10^{-5} mg/kg-day for MA DEP.

After the majority of this document was completed, the U.S. EPA released their October 2003 response to comments document entitled “Disposition of Comments and Recommendations for Revisions to “Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization External Review Draft”. The information contained in this document further supports DEP’s recommended RfD value. The data from the U.S.EPA (2003) response to comments document that support ORS’s choice of endpoints and points of departure to derive an RfD for perchlorate are summarized in Appendix A. The USEPA in conjunction with the Department of Defense, the Department of Energy and the National Aeronautics and Space Administration, requested that the National Academy of Sciences (NAS) conduct a review of perchlorate toxicity. Completion of this review and subsequent finalization of a federal RfD value and associated drinking water limit for perchlorate will likely take several years. MADEP is aware of and is following this review and will address any new scientific information derived from this effort when it becomes available.

Technical work on this MA DEP document was complete through January 2004.

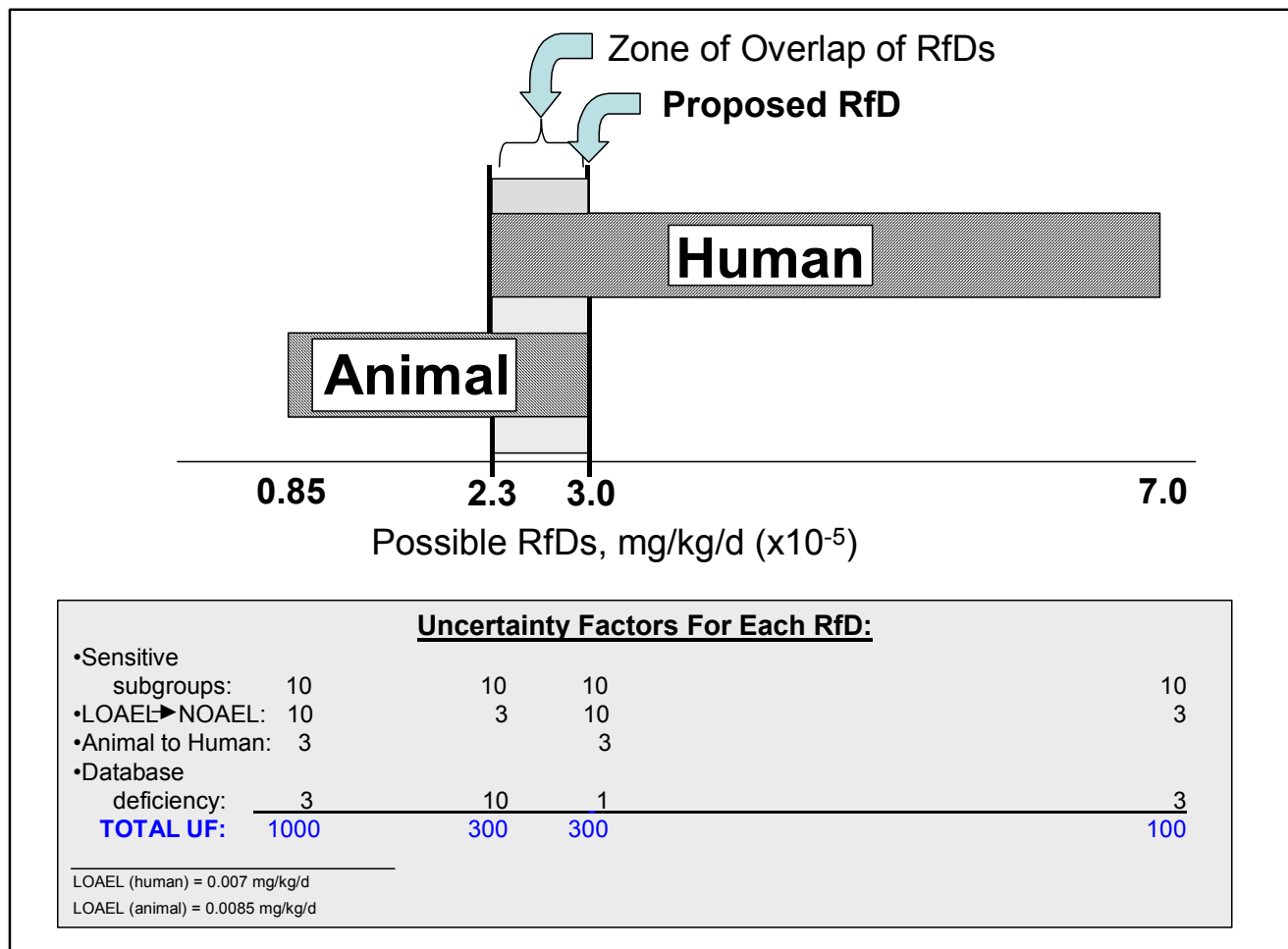


Figure 1. Summary of the RfD Derivation Process and RfD Ranges

1. INTRODUCTION

Ammonium perchlorate is widely used as a component of propellants for rockets, missiles, and fireworks. Perchlorate use has led to contamination of soils, surface and ground waters in many states around the country. This compound is highly mobile in aqueous systems and can persist for many decades under typical ground and surface water conditions (U.S. EPA, 2002).

Perchlorate was first detected in high concentrations in monitoring wells in California in the early 1990s. Since then it has been found more widely around the country. Perchlorate has been detected at levels up to 500 ppb in groundwater at the Massachusetts Military Reservation (MMR) on Cape Cod. A little over a year ago, perchlorate was also detected in the abutting town of Bourne's water supply at concentrations less than 1 ppb. In response, the Bourne Water District (BWD) voluntarily and temporarily shut three affected wells. Since there were no (and still are no) established drinking water standards for perchlorate, in March 2002, the BWD formally requested guidance from MA DEP on perchlorate in drinking water. In order to assist the BWD, DEP toxicologists and risk assessors reviewed available information on the toxicity of perchlorate, including the draft EPA health assessment for perchlorate (U.S.EPA, 2002), which contains a draft reference dose and an associated drinking water limit of 1 ppb for perchlorate. This report as well as other information reviewed indicated that risks to sensitive subgroups, including pregnant women, children and individuals suffering from hypothyroidism, could not be ruled out at perchlorate drinking water concentrations above 1 ppb. As these risks included the potential for serious adverse outcomes, including permanent neurological effects from *in utero* and postnatal exposures, MA DEP provided the BWD with interim advice recommending that these sensitive subgroups be informed when perchlorate concentrations exceed 1 ppb and be advised to avoid consuming the water. MA DPH supported this interim advice and EPA Region 1 issued a statement indicating that the advice was health protective.

At the time of the initial detection in California, the Region 9 Office of the United States Environmental Protection Agency developed a provisional RfD for perchlorate of 0.0001 mg/kg-day in 1992. This was revised to 0.0005 mg/kg-day in 1995. These values were based on a dated acute exposure study conducted by Stanbury and Wyngaarden(1952) in which single doses of potassium perchlorate caused release of iodide (I^-) from thyroids of patients with Graves' Disease, an autoimmune condition that results in hyperthyroidism. The difference in the RfDs stems from the use of different uncertainty factors: 1000 (1992) versus 300 (1995). These RfDs were characterized as provisional because they did not undergo the internal peer review required of such values available on the U.S. EPA's Integrated Risk Information System (U.S. EPA, 2002a). Using the standard default assumptions of 70 kg for body weight and 2L/day for water ingestion rate, drinking water equivalent levels of 4 and 18 $\mu\text{g/L}$ were developed for water using the two RfDs. The range bounded by these two values was provided as EPA's best estimate of perchlorates's RfD. Drinking water equivalent levels by definition do not address exposures beyond ingestion of water. Potential exposures to perchlorate through the food-chain (e.g. from lettuce grown using perchlorate contaminated water) are not considered in the derivation of the 4-18 $\mu\text{g/L}$ range. In part because of the improvements made in instrumentation to detect low concentrations of perchlorate, the provisional RfDs were revisited by a review panel in March 1997. Eight additional new categories of studies to build a robust database that could be used to

assess the health effects of perchlorate were recommended and conducted in an expedited time frame through the partnership and cooperation of the Interagency Perchlorate Steering Committee (IPSC) which included representatives from U.S.EPA, Department of Defense (DOD), the National Institute for Environmental Health Sciences (NIEHS), and affected state, tribal and local government. The U.S.EPA incorporated the data from these studies into an updated assessment which led to the release of its first draft document on perchlorate toxicity in 1998. The final U.S.EPA draft document, which recommended an RfD of 0.00003 mg/kg-day, was released in January of 2002. Using the same standard default assumptions for bodyweight and water ingestion rate as noted previously, a draft drinking water equivalent of 1 µg/L was calculated. The operational derivation of the draft RfD was based on rat neurodevelopmental studies that demonstrated changes in thyroid structure and hormone levels in dams and pups, and alterations in brain morphometry and behavior in pups exposed to perchlorate *in utero* and postnatally at low concentrations. The U.S.EPA (2002) draft document, which has already undergone extensive expert peer and public review, has been forwarded to the National Academy of Sciences (NAS) for further review that is scheduled to be completed in Fall 2004. To help inform decisions regarding site cleanup and responses to drinking water contaminants, MA DEP has developed an RfD value. This reference dose will be used to establish state hazardous waste site cleanup levels for perchlorate under the Massachusetts Contingency Plan (MCP) for site-specific risk assessments and to possibly establish a drinking water standard.

Technical work on this document was current through January 2004.

2. CHEMICAL IDENTITY

Perchlorate (ClO_4^-) is an oxygenated anion with a negative charge that can combine with other cations such as sodium, potassium, ammonium or magnesium to form salts. Its salts, except potassium, are soluble in water and dissociate to form the perchlorate anion. Perchlorate salts are used as rocket fuel oxidizers, explosives, air bag inflators, and in many other industrial and commercial applications (U.S. EPA, 2002).

3. ENVIRONMENTAL CONTAMINATION AND HUMAN EXPOSURE

Ammonium perchlorate has been widely used as an oxidizer in solid propellants for rockets and missiles since the mid-1940s and in fireworks. Because of its short shelf life, the rocket propellant containing perchlorate has been periodically washed out of the United States' missile and rocket inventory to be replaced with a fresh supply (U.S. EPA, 2002). As a result, large volumes of perchlorate have been discarded since the 1950s. Some of this waste has leached into soil, and into aquifers used as drinking water sources. Because of its physical and chemical characteristics, perchlorate can persist in ground and surface waters for many decades (U.S. EPA, 2002).

Agricultural and domestic use of such waters can result in human exposure. The major routes of exposure to perchlorate are ingestion of perchlorate contaminated water and possibly food.

Lettuce irrigated with perchlorate-tainted water in a laboratory setting accumulated 500 times the concentration of perchlorate in the irrigation water (U.S.EPA, 2001). U.S.EPA cautioned that results obtained under laboratory growing conditions could not be directly extrapolated to edible agricultural produce. In another study (Yu, et al. 2003), lettuce, cucumber, and soybean were used to determine the uptake of perchlorate from sand. Significant uptake of perchlorate was observed in all three plants watered with 100 ppb perchlorate and a threshold concentration was reached for cucumber at 150 ppm and for lettuce at 750 ppm. Recently, the Environmental Working Group, a private nonprofit environmental group, sampled lettuce from markets in California. Four of the 22 samples tested contained perchlorate with average concentration of 72 ppb. In addition, researchers from the Institute of Environmental and Human Health at Texas Tech University measured perchlorate in samples of supermarket milk over a range of 1.0 to 6.4 ppb. No other survey or other published data were found on perchlorate levels in raw or processed foods. Breast milk may also be an exposure pathway for infants. Perchlorate was found in higher concentrations in breast milk of treated animals than the serum even at the lowest dose (0.01 mg/kg-day) tested (2000, as cited in CA EPA, 2002). This exposure pathway is of further concern because infants depend on maternal iodide that is secreted into the breast milk during early development. Perchlorate may inhibit the active transport of iodide into the mammary gland at the Sodium (Na)-Iodide-Symporter (NIS). NIS is a membrane-bound protein that is involved in the transport of iodide, and is expressed in the mammary gland during lactation (Tazebay et al., 2000).

Since perchlorate exists in the ionized state in water, its skin penetration is limited. Inhalation exposure from bathing and showering is minimal since perchlorate is not volatile and the droplets produced in the shower are too large to be inhaled (U.S.EPA, 2002).

Perchlorate particles can be suspended in the air and can be inhaled by individuals working in areas where perchlorate is manufactured (Lamm *et al.*, 1999). Although release of perchlorate to the atmosphere is possible during the launching of solid fuel rockets, fireworks and as a result of the open detonation of old rocket fuel, no published data were found on levels of perchlorate in the ambient air.

Soil contamination from past disposal practices of perchlorate or irrigation of soil with perchlorate-contaminated water is possible. Soil runoff could lead to pollution of drinking water sources and leafy vegetables grown in such soils could bioaccumulate perchlorate. In general, the extent to which fruits and other agricultural products in addition to lettuce can accumulate perchlorate is not known. Another source of soil contamination is the use of fertilizers tainted with perchlorate. Nitrate fertilizers from Chile that are derived from caliche ores contain naturally occurring perchlorate. However, because of low usage of Chilean nitrate in the United States, and also because of improved refining processes of these fertilizers, Chilean nitrates could not represent a significant anthropogenic source of perchlorate contamination nationwide, regardless of the perchlorate content (U.S. EPA, 2001).

4. TOXICOKINETICS

4.1 ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION

Human studies have indicated that ingested perchlorate is readily and completely absorbed from the gastrointestinal tract and excreted rapidly primarily via the urine (Eichen, 1929; as cited in Stanbury and Wyngaarden, 1952; Durand, 1938). Absorbed perchlorate is suggested to bind to the NIS protein which is known to translocate iodine into the thyroid and other tissues including the gastric mucosa, mammary gland, and placenta (Dohan et al., 2002). The NIS protein is believed to have about 10 times the affinity for perchlorate than for iodide (Clewett et al., 2003).

While there is no controversy about the ability of perchlorate to inhibit iodide uptake at the NIS, whether or not perchlorate itself is translocated into the thyroid via the NIS is a matter of debate. Eskandari et al. (1997) reported that addition of 500 μM perchlorate to a bathing medium containing iodide abolished the existing inward current in an oocyte with NIS. These authors also reported that whereas iodide and a variety of other anions (ClO_3^- , SCN^- , NO_3^- , Br^- , IO_4^- and BrO_3^-) generated steady-state inward currents in the system that suggest that these anions are transported, perchlorate (ClO_4^-) did not. The results were interpreted to mean that perchlorate is not transported into thyroid cells by the NIS (De la Vieja et al., 2000). Riedel et al. (2001a,b) proposed two potential mechanisms for the action of perchlorate on the thyroid: (1) perchlorate blocks iodide transport but is not translocated into the thyroid cells, and (2) it competes with iodide and is transferred into the cell by NIS at a 1:1 ratio with sodium. Other authors have also reported that perchlorate competes with iodide and is highly concentrated in the thyroid gland (Anbar et al.; 1959; Chow et al. 1969; and Chow and Woodbury, 1970).

The metabolism of perchlorate is not well studied. The urine of four subjects treated with radioactive double-labeled potassium perchlorate ($\text{K}^{36}\text{Cl}^{18}\text{O}_4$) contained $^{36}\text{ClO}_3^-$ and small concentrations of $^{36}\text{Cl}^-$, in addition to $^{36}\text{Cl}^{18}\text{O}_4^-$ (Anbar et al., 1959). These results suggest that some ingested perchlorate may be metabolized. No other studies on the biotransformation of perchlorate were located.

5. TOXICOLOGIC PROFILE

5.1 HYPOTHALAMUS-PITUITARY-THYROID AXIS

The target tissue for perchlorate toxicity is the thyroid gland. One of the primary functions of the thyroid is secretion of two important hormones (thyroxine [T4] and triiodothyronine [T3] whose major effects are to: (1) increase metabolism; and (2) stimulate growth and promote normal development in children (Ganong, 1987; Guyton and Hall, 2000). Perchlorate suppresses thyroid gland function by inhibiting the transport of iodide at the sodium-iodide symporter (NIS), which is a membrane-bound protein involved in the active transport of the iodide ion into the thyroid gland. This action of perchlorate has been the basis for its clinical use in the treatment of hyperthyroidism in Graves' Disease patients. Perchlorate's mode of action was also used to alter metabolic rate in livestock. Since perchlorate-induced hypothyroidism can increase body weight by lowering metabolism, perchlorate has been tested as a feed supplement in livestock. An

increase in body weight of 3-31% was observed in the animals, and the optimum dose for this effect was 2-5 mg/kg-day (Yakimenko et al. 1981, as cited in Burg, 1995).

The major substrates for thyroid hormone synthesis are iodide and tyrosine. Iodine is ingested in a variety of chemical forms. Most ingested iodine is reduced into iodide in the gut and absorbed almost completely (Nath et al., 1992). These dietary-supplied trace levels of iodine are the rate-limiting substrate in the synthesis of the hormones. The thyroid tissue has a special ability to concentrate iodide from the blood. In a normal gland, the iodide concentration can be 30 times the concentration in the blood. When the thyroid gland becomes maximally active, the concentration ratio can rise to as high as 250 (Guyton and Hall, 2000). The normal human thyroid daily secretes about 80 µg (103 nmoles) of T4 and 4µg (7 nmoles) of T3. While T4 is produced only in the gland, about 80% of T3 is formed in the peripheral tissues by enzymatic deiodination of T4. Once the thyroid hormones are secreted into the bloodstream they are bound to three plasma proteins: albumin, thyroxine-binding prealbumin (TBPA) or transthyrein and thyroxine-binding globulin (TBG). Of the three proteins, TBG binds T4 with high affinity and T3 with lower affinity. The rat T4 and T3 are bound to prealbumin or albumin with weaker affinities when compared to thyroid hormone binding to TBG in humans (U.S. EPA, 2002).

In humans, the normal total plasma T4 level is about 8 µg/dL and that of T3 is about 0.15 µg/dL. The free T4 (fT4) level is about 0.002 µg/dL (0.025%) with a biologic half-life of about 6 - 7 days. The remaining 99.98% of the plasma T4 is protein bound. Of the 0.15 µg/dL T3, only 0.3 ng/dL (0.2%) is free and the remaining 99.8% is protein bound. T3 acts more rapidly than T4 and is 3 to 5 times more potent than T4. It is these free hormones, mainly T3, that enter the cell for biological action. T4 and T3 are metabolized mainly in the liver and undergo sulfate and glucuronide conjugation. These metabolites enter the bile and some of the iodine in them is reabsorbed.

To maintain the right concentration of thyroid hormones, a specific negative feedback mechanism operates through the hypothalamic-pituitary-thyroid axis (Figure 2). The hypothalamus stimulates the pituitary through thyrotropin releasing hormone (TRH) to produce thyroid stimulating hormone (TSH) that in turn stimulates the thyroid gland to produce and release T4 and T3. The pituitary gland responds to the levels of circulating T4 and T3. When these hormone levels are reduced, the pituitary gland is prompted to deliver more TSH to the thyroid for the synthesis of more hormones. The negative feedback effect of thyroid hormones on TSH secretion may be exerted in part at the hypothalamic level but must be mainly on the pituitary since T3 and T4 block the increase in TSH secretion produced by TRH (Ganong, 1987). If pituitary stimulation persists, the thyroid cells increase in size (hypertrophy) and number (hyperplasia) leading to thyroid enlargement (goiter) and probably neoplasia.

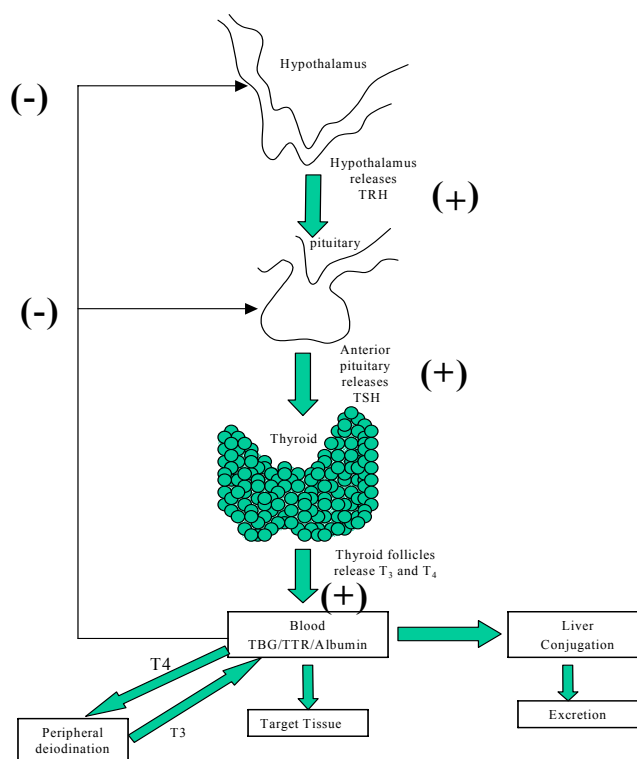


Figure 2. Hypothalamus-Pituitary-Thyroid Axis

Although perchlorate's ability to cause an iodine-deficient thyroid is well established, the epidemiological database on its toxicity is limited. There are however, a number of epidemiological studies conducted in people who live in iodine-deficient geographic areas. These studies have demonstrated that the most sensitive subgroups for iodine deficiency are pregnant mothers, their fetuses, children and hypothyroid people.

6. IODINE DEFICIENCY: HUMAN STUDIES

6.1 PREGNANT WOMEN

Pregnancy is a major factor for a stressed thyroid gland in women. A normal thyroid is faced with three challenges during pregnancy: (1) the major T₄ transporting protein (thyroglobulin) is increased in response to a high estrogen level. Consequently, the thyroid has to increase T₄ production to maintain a stable T₄/thyroglobulin ratio; (2) the placenta releases various thyroid stimulating factors, mainly the human chorionic gonadotropin (hCG) which results in lower serum TSH concentration and increased thyroid volume which is consistent with a TSH-like effect of hCG; and (3) there is decreased availability of iodide for the maternal thyroid due to increased renal clearance and losses to the feto-placental complex during gestation (Glinioer et al., 1990). These changes in pituitary and thyroid hormone levels and thyroid size are

exacerbated even by mild iodine deficiency (iodine intake of $< 100 \mu\text{g/day}$). Moderate and severe iodine deficiencies are believed to occur when iodine intakes are less than $50 \mu\text{g/day}$ and $20 \mu\text{g/day}$, respectively.

The median urinary iodine concentration in iodine sufficient people should be greater than $10 \mu\text{g/dL}$. Two National Health and Nutrition Examination Surveys [NHNES I (1971-1974) and [NHNES II (1988-1994) suggest that some women of childbearing age in the U.S. may have relatively low iodine intake as determined by urinary iodine concentrations of $<5 \mu\text{g/dL}$ (Hollowell et al 1998). In severe iodine-deficient populations the urinary iodine levels are reported to be less than $2 \mu\text{g/dL}$ (Todd, and Bourdoux, 1991).

Glinioer et al. (1990) studied pregnant women living in a marginally iodine-deficient area ($50 - 70 \mu\text{g/day}$ iodine intake). All subjects were evaluated clinically and determined to be without detectable thyroid abnormality at the beginning of the study. The authors found an overall reduction in the T4/T3-binding globulin ratio, with lower free T4 and T3 levels as pregnancy progressed. Most women exhibited hypothyroxinemia (higher T3/T4 ratio), probably indicating preferential T3 secretion. When thyroid enlargement was compared between pregnant and non-pregnant women with low iodine intake (urine iodine levels of $4.29 \mu\text{g/dL}$), the size of the gland was larger in pregnant women when compared to non-pregnant controls. There was no difference in goiter size between pregnant women and non-pregnant controls living in iodine-sufficient areas (urine iodine levels of $6.91 \mu\text{g/dL}$) (Crooks et al. 1967). Change in thyroid enlargement related to pregnancy was also reported by Levy et al. (1980); Smyth et al. (1997); Caron et al., 1997; Kung et al. (2000); Rotondi et al. (2000); and thyroid hormone changes were reported by Klien et al. (1991) and Caron et al. (1997).

Studies conducted in marginally iodine deficient areas in Europe demonstrated that, in pregnant women supplemented with iodine, thyroid volume did not change significantly when compared with pregnant women who did not receive iodine supplementation (Romano et al., 1991; Pederson et al., 1993). Thyroid stimulation associated with pregnancy and leading to preferential T3 secretion by the thyroid was suppressed after potassium iodide and T4 treatment (Glinioer et al., 1995).

There is now an established link between low dietary iodine intake by pregnant mothers and a spectrum of abnormalities in offspring. Degrees of iodine deficiency are: mild ($<100 \mu\text{g/day}$ iodine intake), moderate ($<50 \mu\text{g/day}$ iodine intake) and severe ($<25 \mu\text{g/day}$ iodine intake). Severe iodine deficiency during pregnancy can cause perinatal death and cretinism (WHO, 1994; as cited in Hollowell *et al.*, 1998). Two subtypes of endemic cretinism have been reported, neurologic cretinism and myxedematous cretinism. Neurologic cretinism is more common and is characterized by delayed growth of long bones, neurological complications such as deaf mutism, mental retardation, and bilateral paralysis of both sides of any part of the body. Goiter is sometimes associated with this illness. The other type of cretinism is myxedematous which is less common and with less severe mental retardation but with all the clinical symptoms of chronic hypothyroidism. Neurologic damage (retardation and abnormalities of brain and physical development) in the absence of neonatal hypothyroidism has been postulated to be due to maternal hypothyroxinemia early in gestation (Burrow *et al.*, 1994). Even mild iodine deficiency ($<100 \mu\text{g/day}$) during pregnancy has been linked to retarded physical development and reduction

of intelligent quotient (IQ) of the offspring (Bleichrodt et al., 1989; Vermiglio, et al., 1990; Bleichrodt and Born, 1994).

6.2 OFFSPRING OF IODINE-DEFICIENT MOTHERS

Chemicals like perchlorate that inhibit the availability of iodine to the thyroid gland and reduce the synthesis of thyroid hormones have the potential to produce the same health effects observed in iodine-deficient conditions. The neurological abnormalities induced by iodine deficiency in children born to iodine-deficient mothers are discussed in this context in the following section.

It is known that brain development occurs during discrete windows of time during gestation (Figure 3), and inappropriate levels of thyroid hormones in definitive periods can produce permanent damage, the nature of which depends upon the timing and magnitude of the insult. Maternal thyroid function during early pregnancy is an important determinant of early fetal brain development because the fetal thyroid is unable to produce any T4 before 12-14 weeks of gestation (Pop et al., 1999). Maternal serum T4 passes through the placenta and is converted to T3 in the fetal brain, which is the predominant form of the hormone that binds to thyroid receptors. T3 generated by enzymatic deiodination of T4 in the fetal brain itself is necessary for the normal development of the various regions of the brain, specifically the cerebral cortex, the extrapyramidal system, and the cochlea (Porterfield, 2000). It is reported that normal levels of maternal T3 do not seem to prevent the damage caused to the fetus by low supply of T4 (CA EPA, 2002). To maintain normal supply of T4 the pregnant and lactating mother should have an iodine intake of 200 µg/day (WHO/UNICEF/ICCIDD, 2001).

Epidemiological studies performed in iodine-deficient populations strongly suggested that an early maternal hypothyroid state increases the risk of neurodevelopmental deficit in the fetus whether or not the mother is clinically hypothyroidic (Lavudo-Autrit et al. 2003). Maternal hypothyroidism caused by marginal iodine deficiency in humans (<100 µg/day iodine intake) was associated with lowered mental development in offspring manifested as a deficit in global intelligent quotient (IQ) of 5-13 points, defective visual perception and altered motor activity (Bleichrodt et al. 1989; 1994; Vermiglio, et al., 1990; Fenzi et al., 1990; Vitti et al., 1992; Azizi et al., 1993; Shresta, 1994; Aghini-Lombardi et al., 1995; Tiwari et al., 1996; Van den Briel et al, 2000). Glorieux et al. 1985; 1988; Tillotson et al., 1994; Vermiglio et al., 1990) (Table 2).

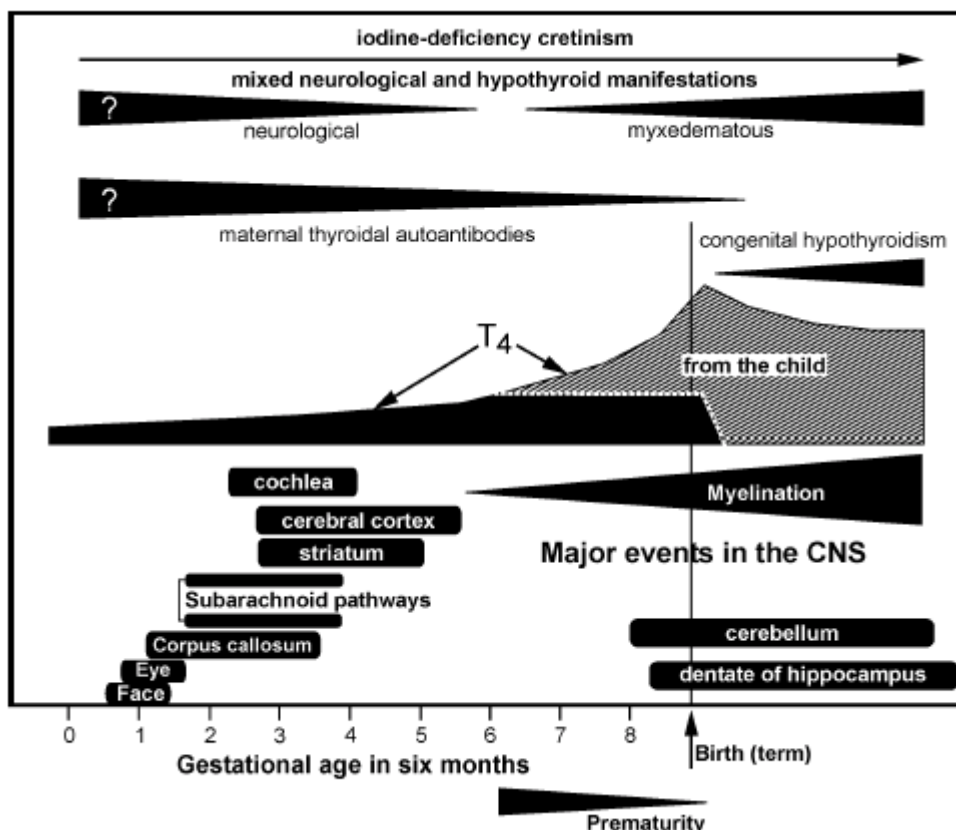


Figure 3. Approximate Timing of Major Insults to the Brain Resulting From Hypothyroxinemia, Superimposed on Major Neurodevelopmental Events

Conditions resulting in early maternal hypothyroxinemia, combined with later impairment of the fetal thyroid, are the most damaging, with central nervous system (CNS) damage that is irreversible at birth. The most frequent cause is maternal iodine deficiency and the presence of maternal autoimmune thyroid disorders. Unless iodine deficiency is also present, the CNS damage in congenital hypothyroidism is preventable by early postnatal treatment because the normal maternal thyroxine level has avoided damage to the brain until birth. However, normal maternal concentrations of T3 with low T4 do not protect the fetal brain because of its dependence on intracerebral regulation of local T3 availability by deiodinating pathways using T4 as a substrate. Interruption of the contribution of maternal T4 in premature infants with an immature thyroid may also underlie their increased risk of neurodevelopmental problems. The question mark indicates that we do not know whether very early CNS development, corresponding to a period when the general morphogenesis of the prosencephalon (neurulation and segmentation) is being determined, is thyroid hormone sensitive or not (from Morreale de Escobar *et al.*, 2000; as cited in U.S.E EPA, 2002).

Women are more prone to suffer from hypothyroidism related to autoimmune disorder than men. An estimated 4.6% of the United States population has hypothyroidism (0.3% clinical and 4.3% subclinical). Immune-related hypothyroidism in women of childbearing age could result in neurodevelopmental deficit in their offspring. It is not known whether this effect could be exacerbated by iodine deficiency or by exposure to chemical agents that interfere with thyroid activity.

Maternal hypothyroidism or small decrements in maternal fT4 (free T4) levels are related to impaired brain development. Pop et al. (1999) studied neurodevelopment in iodine-sufficient areas and reported that children of women with fT4 levels below the 5th (< 9.8 pmol/l, n = 11) and 10th (< 10.4 pmol/l, n = 22) percentiles at 12 weeks' gestation had significantly lower scores on the Bayley Psychomotor Developmental Index (PDI) scale at 10 months of age, compared to children of mothers with higher fT4 values (t-test, mean difference: 14.1, 95% confidence interval (CI): 5.9-22 and 7.4, 95% CI: 1.1-13.9, respectively) and concluded that low maternal plasma fT4 concentrations below the 10th percentile at 12 weeks of gestation were significant risk factor (RR 5.8) for impaired psychomotor development in the offspring.

Table 2. Neuropsychointellectual Deficits in Infants and School Children in Conditions of Mild to Moderate Iodine Deficiency

Regions	Findings	References
Spain	Lower psychomotor and mental development than controls	Bleichrodt et al., 1989
Sicily	Low perceptual integrative motor ability, neuromuscular and neurosensorial abnormalities	Vermiglio, et al., 1990
Tuscany	Low verbal IQ, perception,	Fenzi et al., 1990
Tuscany	Lower velocity of motor response to visual stimuli	Vitti et al., 1992 Aghini-Lombardi et al., 1995
India	Lower capacity of learning	Tiwari et al., 1996
Iran	Retardation in psychomotor development	Azizi et al., 1993
Malawi	Loss of 10 IQ points as compared to iodine- supplemented controls	Shrestha, 1994
Various locations	Met analysis of 21 iodine deficiency studies -loss of 13 IQ points	Bleichrodt and Born, 1994
Benin	Loss of 5 IQ points as compared to controls supplemented with iodine for one year	Van den Briel et al., 2000

Haddow et al. (1999) investigated serum of 2516 pregnant women in Maine and identified 47 women with serum thyrotropin (TSH) levels above the 99.7 percentile and 15 women between the 98th and 99.6th percentile of the value in all the pregnant women. T4 levels were also low in these women. The authors found neuropsychological deficit in the children born to mothers with this altered thyroid pituitary function when compared to mothers with normal thyrotropin and T4 levels. The authors concluded that even mild, and probably asymptomatic, hypothyroidism in pregnant women can affect intelligence, attention, linguistic ability, reading ability, school and visual-motor performance and demonstrated that such effects are occurring in U.S population. Klein et al (2001) also found decreased IQ in children associated with maternal hypothyroidism.

Others (McCarrol et al., 1976; Burrow et al., 1978; Messer et al., 1990; and Liu et al., 1994) did not find an association between fetal hypothyroidism and neurodevelopmental impairment in fetuses who had been exposed to antithyroid drugs such as carbimazol, propylthiouracil, or thiamazole. However, these studies are limited as they have relatively small sample sizes and dosages and the timings of the treatments were not known in many cases. In one study (Liu et al., 1994), mothers were treated with T4 at 13 weeks of gestation that would have prevented adverse neurological effects in the fetuses.

6.3 IODINE DEFICIENCY AND THYROID CANCER

There is a tendency for a higher incidence of cancers in the autopsy material from endemic areas with iodine deficiency in the diet. The relationship of thyroid cancer and iodine deficiency has often been debated.

Pettersson et al. (1996) studied the regional pattern of thyroid cancer incidence in relation to iodine intake and iodination in Sweden and found that residents in iodine deficient areas had a two-fold increase in follicular cancer compared to residents in iodine sufficient areas. Iodine supplementation was accompanied by a change in the epidemiological pattern of thyroid cancer with an increased prevalence of occult papillary cancer discovered at autopsy (Vigneri et al. 1998; Williams, 1985). Monitoring of the incidence of cancer in Switzerland following iodine supplementation showed that the incidence of thyroid cancers steadily decreased from 2 to 3 per 100 000 in 1950 to 1 to 2 per 100 000 in 1998 i.e., during a period when iodine intake increased and reached an optimum value (Levi et al., 1991). A comparative study of pre- and post-iodination biopsies of thyroid tumors demonstrated that the frequency of neoplastic lesions significantly decreased and the ratio of papillary/follicular carcinoma increased with iodination.

6.4 EXPERIMENTAL STUDIES

When rats were fed iodine-deficient diets like those consumed by people in areas with endemic cretinism, lower serum T4 and T3 levels, and neonatal goiter were discovered in their fetuses. The neonates also had reduced brain weights and increased radiolabeled iodide uptakes. The density of the brain cells was increased in the cerebral hemisphere. The cerebellum showed delayed disappearance of the external granular layer (Hetzel et al., 1979; McMichael et al., 1980; Zhong et al 1983; Li et al. 1985).

Marmosets fed diets deficient in iodine had offspring with sparsity of hair growth, enlarged thyroid glands, reduced plasma T4 levels (in both mother and newborn). There was significant reduction in brain weight in newborns from the second pregnancy. Cell numbers and weight of cerebellum were also significantly reduced (Mano et al., 1987).

Severe physical damage and brain damage were also observed in sheep fed iodine-deficient diets. There was reduced weight, absence of wool growth, goiter, deformation of the skull, and delayed maturation in the fetus. Reduced plasma T4, thyroid hyperplasia, and decreased brain weight were also observed. The cerebellum showed delayed disappearance of the external granular layer (Potter et al. 1981).

In conclusion, the above peer-reviewed data have demonstrated that the most sensitive subgroups to decreased iodine intake are hypothyroid and iodine-deficient pregnant women and their offspring. An array of U.S.EPA and Department of Defense (DOD) sponsored studies that address the potential toxicities of perchlorate exposure during various critical life stages from the developing fetus through adult and reproductive stages were conducted to see if perchlorate-induced iodine deficiency could similarly affect the identified sensitive subpopulations. These and other relevant studies are reviewed in section 7.

7. PERCHLORATE TOXICITY

7.1 HUMAN STUDIES

7.1.2 Death

An estimated lethal dose in humans upon acute exposure is 214 mg/kg (Von Burg, 1995).

7.1.3 Systemic Toxicity

Historically, potassium perchlorate has been used to treat Graves' Disease in humans, and most of the prior data on perchlorate effects in humans are in patients with this disease. Graves' disease is an immune disorder that is characterized by hyperthyroidism. Graves' Disease, patients have immunoglobulins in their blood that bind to TSH receptors on the thyroid cells and act like TSH leading to a hyperthyroid state. Perchlorate inhibits the excessive synthesis and secretion of thyroid hormones by inhibiting the uptake of iodide into the thyroid and the discharge of accumulated iodide in the gland.

Two hundred patients with Graves' Disease were administered 9 to 14 mg/kg-day perchlorate. The adverse effects observed in these patients were one case of skin rash and three cases of nausea (Crooks and Wayne, 1960). In other groups of 10 patients given 21 mg/kg-day and 40 patients given 29 mg/kg-day, five cases of skin rash, two cases of nausea, and one case of agranulocytosis occurred. Leukocyte counts returned to normal in the patient with the agranulocytosis when perchlorate treatment was stopped. The durations of treatment in the studies were not specified. In other studies, Graves' Disease patients treated with perchlorate doses ranging from 6 to 14 mg/kg-day developed fatal and nonfatal agranulocytotic leukopenia, and fatal aplastic anemia (Fawcett and Clarke, 1961; Hobson, 1961; Johnson and Moore, 1961; Southwell and Randal 1960; Barzilai and Sheinfeld; 1966; Sunar, 1963).

Twenty-four Graves' Disease patients were treated with 9 to 17 mg/kg-day perchlorate for at least 11 weeks with a few as long as 52 weeks. Two patients developed gastrointestinal problems. In one patient, these effects occurred at 9 mg/day (Godley and Stanbury, 1954). A single female Graves' disease patient who was treated with about 3 mg/kg-day perchlorate for 22 years developed no adverse effects (Connell, 1981). No LOAELs/NOAELs were identified in the human studies because of the diseased state of test subjects.

7.1.4 Endocrine Toxicity

U.S.EPA (1998) proposed a model based on mapping the events of the mode of action for perchlorate as shown in Figure 4. The key event was identified as the inhibition of iodide uptake at the NIS, followed by decreases in thyroid hormones and increases in TSH. Both the potential neurodevelopmental and neoplastic sequelae of this perturbation in thyroid hormone economy were proposed as downstream adverse health outcomes. The conceptual model was endorsed by the external peer review panel in 1999 (Research Triangle Institute, 1999, as cited in U.E.EPA, 2002). Additional studies were recommended to reevaluate indications of developmental and

neurodevelopmental in rats for effects observed in the 1998 database. Delineating the continuum of histopathological changes in the thyroid was also recommended. The results of all studies in the perchlorate testing strategy and other pertinent studies from the literature on perchlorate toxicity are discussed below.

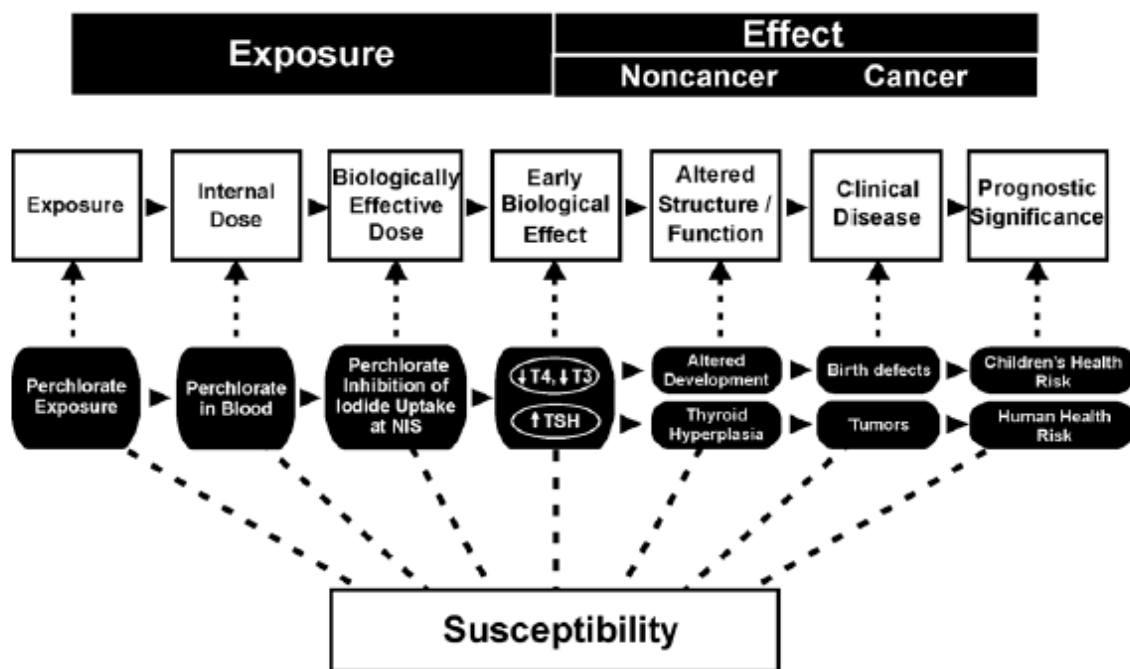


Figure 4. Mode-of-action model for perchlorate toxicity proposed by the U.S. EPA (U.S. Environmental Protection Agency, 1998, 2002, 2003).

Perchlorate interferes with iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS) present in various tissues such as the thyroid, gastrointestinal (GI) tract, skin, placenta, and mammary gland. Schematic shows the exposure-dose response continuum considered in the context of biomarkers (classified as measures of exposure, effect, and susceptibility) and level of organization at which toxicity is observed. The model maps the toxicity of perchlorate on this basis by establishing causal linkage or prognostic correlations of precursor lesions (Adopted from U.S.EPA, 2003).

7.1.4.1 Thyroidal Iodide Uptake Inhibition and Iodide Release

Stanbury and Wyngaarden (1952) studied the effects of perchlorate on the discharge and uptake of iodide by the thyroid in 8 Graves' Disease patients. A single dose of 1.4 mg/kg-day perchlorate caused complete discharge while a single dose of 0.14 mg/kg-day caused 50% release of stored iodide from the thyroid gland of Graves' Disease patients. Potassium perchlorate doses as low as 0.04 mg/kg-day caused detectable but incomplete release of iodide from the thyroid of these patients. These studies were conducted using drugs (either 1-methyl-2-mercaptoimidazole or propylthiouracil) that may enhance the release of iodide in the thyroid.

gland by preventing the oxidation of iodide ion to iodine and thyroid hormone synthesis. Moreover the subjects used were diseased individuals. Inhibition of iodide uptake also occurred in patients without either 1-methyl-2-mercaptoimidazole or propylthiouracil pretreatment receiving 1.4 mg/kg-day potassium perchlorate. In addition, Stanbury and Wyngaarden (1952) reported that uptake of labeled iodide into the thyroid glands of two patients with Graves' Disease was significantly inhibited for as long as 6 hours when 1.4 mg/kg-day potassium perchlorate was given orally one hour prior to the administration of the labeled iodide. These data are viewed inadequate to establish a LOAEL or a NOAEL.

Twenty-four Graves' Disease patients were treated with total doses of 600 to 1,200 mg/day (8.6 to 17 mg/kg-day) of perchlorate for at least 11 weeks with a few as long as 52 weeks. In thirteen patients, uptake of radioactive iodine by the thyroid both before beginning perchlorate therapy and within two weeks after medication had begun was determined. The mean control uptake was 77.5 percent, with a range from 60.7 to 108 percent. The mean uptake during perchlorate therapy was 15.9 percent, with a range from 3.4 to 38.8 percent (Godley and Stanbury, 1954). In five normal healthy volunteers treated with 9.7 mg/kg-day perchlorate for eight days, iodide uptake was completely blocked and the excretion of non-thyroxine iodine was increased 65% above background (Bürge *et al.*, 1974).

Two clinical studies performed at low perchlorate doses (Greer et al, 2002, and Lawrence et al., 2001) were identified and are discussed in the following section.

Nine healthy adult male subjects with no thyroid problems and high iodine intakes were treated with 0.14 mg/kg-day perchlorate for 14 days. Highly significant decreases (about 34%, 39%, 41% of baseline, at 4, 8, and 24 hour post-dose respectively) in radioactive iodide uptake (RAIU) were noted (Lawrence et al., 2001). RAIU was significantly higher than baseline two weeks after perchlorate was discontinued at 4, 8, and 24 hour (Table 3). In this study the dietary iodine levels were high as determined from the urine iodine concentrations. Since perchlorate and iodine compete for the same receptor site at the NIS, high concentration of iodine will attenuate the inhibitory effect of perchlorate. In this study serum perchlorate concentrations were similar at 7- and 14-day exposure periods, suggesting that perchlorate may not bioaccumulate. The LOAEL for RAIU inhibition identified in this study was 0.14 mg/kg-day. In a subsequent study, using 0.04 mg/kg-day, no change in RAIU was observed. However, U.S.EPA (2002) stated the QA/QC data were not available for this study and also, there was variability of urine and serum perchlorate results, potentially due to an unstructured drinking water regimen.

Table 3. Thyroid ¹²³I Uptake Measured Before, During and After 14-Day Ingestion of 0.14 mg/kg-day Perchlorate (Lawrence et al., 2000)

Time (Hours)	Baseline Thyroid ¹²³ I Uptake (% of dose)	Thyroid ¹²³ I Uptake 14 Days on Perchlorate (% of Dose)	Thyroid ¹²³ I Uptake 14 Days after Perchlorate was Discontinued (% of dose)
4	12.5 ± 1.3	8.2 ± 0.7*	16.6 ± 2.4**
8	17.3 ± 1.9	10.6 ± 1.0*	21.9 ± 2.8**
24	23.6 ± 2.6	14 ± 1.6*	27.1 ± 3.3***

* p<0.01 different from baseline

** p< 0.01 different from baseline

*** p<0.05 different from baseline

Greer et al. (2002) administered perchlorate in drinking water at 0.007, 0.02, 0.1, or 0.5 mg/kg-day to 37 iodine sufficient healthy male and female volunteers for 14 days, and measured inhibition of radioactive iodide (^{123}I) (RAIU) uptake by the thyroid at different time points. Significant RAIU inhibition was observed in all groups except the lowest dose (0.007 mg/kg-day) (Table 4). However, the low dose group was comprised of only 7 subjects, the relative uptake values exhibited considerable variability in the estimated mean uptake inhibition value (Table 4). The authors, however, estimated the NOAEL to be 0.007mg/kg-day. U.S.EPA (2002) statistically evaluated whether the 0.007 mg/kg-day treatment group had sufficient sample size to detect a difference of the magnitude observed in the other dose groups and found the power to be very low to detect differences between baseline and treatment values.

Table 4. 24-Hour Radioiodine Uptake Inhibition Data (Greer et al., 2002)

Perchlorate Dose mg/kg-day	Number of Subjects	Radio Iodide Uptake Expressed as Percent of Baseline
0.007	7	98.2 ± 8.3
0.02	10	83.6 ± 4.1
0.1	10	55.3 ± 3.9
0.5	10	32.9 ± 3.8

7.1.4.2 Effects on Thyroid and Pituitary Function and Thyroid Structure

Five healthy male volunteers were pretreated with 200 µg/day iodine for four weeks before perchlorate exposure. Iodine exposure was discontinued, and the volunteers were given about 13 mg/kg-day of potassium perchlorate for another four weeks. Total serum T3, T4 and free T3 (fT3) levels and thyroid gland volume were not altered due to perchlorate treatment. However, serum free T4, and intrathyroidal iodine concentration levels were significantly reduced, whereas thyroglobulin serum levels were almost doubled (Brabant et al., 1992), suggesting that the short-term treatment of healthy iodine sufficient individuals with perchlorate can stress thyroidal and pituitary function. The LOAEL in this study was 13 mg/kg-day. In a follow-up study, Brabant *et al.* (1994 as cited in U.S. EPA, 2002) repeated the earlier studies with perchlorate treatment lasting longer than 4 weeks and reported that thyroid volumes increased in all subjects although TSH levels did not increase as expected.

In the Greer et al. (2002) study, as in the Brabant et al. (1992) study, TSH levels were significantly decreased at the highest dose tested (0.5 mg/kg-day). Brabant et al. (1992) suggested the decrease in TSH is due to higher sensitivity of the thyroid to TSH in the early adaptation to iodine depletion. These studies suggest that short-term treatment even with low doses of perchlorate can modify the hypothalamus-pituitary-thyroid feedback mechanism in humans.

7.1.5 Developmental Toxicity

Eight new ecological studies have been conducted since 1999. Most of these studies are on the developmental effects of perchlorate. The U.S.EPA (2002; 2003) has extensively reviewed and critiqued these studies and concluded that these studies are inadequate to be used as a basis for an RfD derivation for perchlorate. For detailed review of these studies readers are referred to

above cited U.S. EPA publications. ORS scientists and the joint advisory committee have also concluded that these studies, due to a number of serious limitations, do not provide data useful to establishing an RfD for perchlorate. Since one of these studies conducted by Crump et al. (2000) has received wide attention it is discussed briefly in the following section.

Crump et al. (2000) studied 162 school children (mean age 7.5 years) and 9784 newborn babies in three Chilean cities that have different concentrations of perchlorate in the drinking water: Taltal (100 to 120 µg/L), Chanaral (5-7 µg/L) and Antofagasta (<5 µg/L). Urine iodine concentrations were in the normal range in the school children. Mean levels of TSH, T4, and T3 in the school children were similar among all three cities. High risk of goiter was observed in Chanaral (26.5%), Taltal (23.3%) and Antofagasta (17%). Goiter prevalence was higher in Chanaral (5-7 µg/L) because this city was believed to have had iodine deficiency. The high background goiter prevalence in Antofagasta cannot be explained. Also, in this study, children with lifelong residence in Taltal were five times more likely to report a family history of thyroid disease compared to lifelong residents in Antofagasta (Table 5).

Table 5. Odds Ratio for Association Between Self-Reported Family History of Thyroid Disease¹ Among School Children and City of Residence² (Crump et al., 2000)

City	Concentration of perchlorate in drinking water (µg/L)	School Children With Less Than lifelong Residence (n=162)		School Children With Lifelong Residence (n=127)	
		Odds Ratio	95% confidence interval	Odds Ratio	95% confidence interval
Antofagasta	<5	1.0	-	1.00	-
Chanaral	5-7	0.89	0.25-3.19	1.04	0.21-5.09
Taltal	100-120	3.35	1.19-9.38	4.97	1.29-19.17

¹Direct relative (parent, sibling, grandparent, great-grandparent, aunt, uncle, or cousin)

²Adjusted for age, sex and urinary iodine; excluded one child with autoimmune disease

Table adopted from (Crump et al., 2000)

Crump et al. (2000) also performed neonatal thyroid assessment. Linear regression comparison showed that mean log TSH values of newborns were significantly lower in Taltal than the other two cities. However, for the group of newborns sampled for day one and two, the mean and median values of TSH levels of Taltal were higher than those of Chanaral and Antafagosta. It is hard to reconcile these divergent results in TSH values and make any conclusion. Besides, the mean and median ages (day of life) at screening were different for the 3 cities to make appropriate comparisons of TSH values. This study has scientific and technical limitations that it is considered inadequate by ORS to be used as a basis for RfD derivation for perchlorate. The limitations of this study are further discussed in Section 8.2.

7.1.6 Neurotoxicity

No studies were identified on the neurotoxicity of perchlorate in humans. However, neuropsychological abnormalities (See Section 6.2) observed in children living in iodine-deficient areas are relevant to perchlorate toxicity due to its mechanism of action.

7.1.7 Immunotoxicity

There are no *in vivo* studies on the immunotoxicity of perchlorate in humans. In an *in vitro* study, Weetman *et al.* (1984) investigated the effect of perchlorate on human T and B cell responses to mitogen, and concluded that perchlorate has significant immunosuppressive activity at pharmacologically relevant concentrations that is not due to simple cytotoxicity (assessed by ethidium bromide/acridine orange fluorescence). These results warrant immunotoxicity studies in more sensitive species as studies in mice did not show similar immunosuppressive effects.

7.1.8 Carcinogenicity

There are no human data on the carcinogenicity of perchlorate. However, based on its proposed mode of action, carcinogenic investigations performed in iodine-deficient populations can be viewed as relevant studies for perchlorate exposure. There tends to be a higher incidence of cancers in the autopsy material from iodine-deficient areas (Wahner *et al.*, 1966; Fierro-Benitez, 1973) although the relationship between iodine deficiency and thyroid cancer is often disputed.

Pettersson *et al.* (1996) studied the regional pattern of thyroid cancer incidence in relation to iodine intake and iodination in Sweden and found that residents in iodine deficient areas had higher rates of thyroid follicular cancer compared to residents in iodine-sufficient areas. Williams *et al.* (1977) found that the incidence of papillary and follicular thyroid cancer were separately influenced by dietary iodine with papillary cancer five times higher and follicular cancer less frequent in Iceland (an area of high iodine) than in Northeast Scotland (an area of low iodine). Vigneri *et al.* (1998) and Williams (1985) reported that iodine supplementation changed the pattern of thyroid cancer with an increased prevalence of occult papillary cancer discovered at autopsy instead of follicular type cancers. Monitoring the incidence of thyroid cancer in Switzerland, Levi *et al.* (1991) reported that thyroid cancer prevalence steadily decreased with iodine supplementation. In addition, Stadel (1976) has reported that geographic differences in the rates of breast, endometrial, and ovarian cancer appear to be inversely correlated with dietary iodine. A low dietary iodine may produce a state of increased effective gonadotrophin stimulation, which in turn may produce a hyperestrogenic state characterized by relatively high production of estrogen and estradiol. This altered endocrine state may increase the risk of breast, endometrial, and ovarian cancer.

7.1.9 Genotoxicity

No data were found on the genotoxicity of perchlorate in humans. However, studies in animals and lower organisms suggest that perchlorate may not be genotoxic (ManTech Environmental Technology, 1998; Zeiger, 1998), and the mechanism for perchlorate-induced tumor formation may be due to perturbation of the hypothalamus-pituitary-thyroid axis.

7.2 ANIMAL STUDIES

7.2.1 Death

The oral LD₅₀ (the lethal dose in 50% of test animals) for ammonium perchlorate ranged from 750 to 4200 mg/kg-day in various species. In mice treated with various salts of perchlorate by intraperitoneal injection, the LD₅₀ ranged from 29 to 1500 mg/kg. In general, autopsy results of the acute studies demonstrated damage to the stomach wall and intestine, pulmonary edema, and vascular dilation and congestion of the spleen, brain and sinuses (CA EPA, 2002).

7.2.2 Systemic Toxicity

In the reviewed animal studies, the observed adverse effects of perchlorate are perturbation of thyroid and pituitary hormone regulation and alteration of thyroid and brain morphometry. The only study on systemic effects was that reported by Shigan (1963). In rats treated with 650 mg/kg-day for one month, no noticeable adverse effects were observed, however, when rats were treated with 190 mg/kg-day perchlorate for three months, regulation of the involuntary nervous system was affected, and the protein fraction in the serum and the liver's ability to produce glycogen for carbohydrate storage were altered.

7.2.3 Endocrine Toxicity

7.2.3.1 Thyroidal Iodide Uptake Inhibition

Since the historical data on perchlorate suggested that this compound interfered with iodide uptake and release in the thyroid gland of hyperthyroid patients, animal studies were conducted to see if perchlorate could exert the same effect in animals with normal thyroid function. In studies using Sprague-Dawley rats, which are briefly summarized below, perchlorate also inhibited iodide uptake by the thyroid. One experiment suggested that the degree of inhibition decreases with time, perhaps indicating a degree of adaptation.

Yu *et al.* (2000), working with the United States Air Force and U.S. EPA, investigated the inhibitory effects of perchlorate on thyroidal iodide uptake in rats. Sprague-Dawley male rats (6 animals per dose and time point) were injected with perchlorate at 0, 0.01, 0.1, 1 or 3 mg/kg. At 2 hr post dosing, the rats were challenged with 33 µg/kg ¹²⁵I with carrier by intravenous injection and euthanized at various time points post dosing. Statistically significant thyroidal iodide uptake inhibition was found in the 1 and 3 mg/kg perchlorate dose groups at 2, 6, and 9 hr time points. In addition, significant inhibition was also observed in the 0.1 mg/kg dose group at the 9 hr time point.

In a follow-up study, Yu *et al.* (2000) exposed groups of male Sprague-Dawley rats (6 animals per dose) to perchlorate in drinking water with target concentrations of 0, 1, 3, and 10 mg/kg-day continually for 1, 5, or 14 days. At the end of days 1, 5, or 14, rats were challenged once with 33 µg/kg ¹²⁵I with carrier and euthanized 2 hr later. Blood and thyroid glands were collected for analyses. A dose-related inhibition in iodide uptake was noted in the one-day

treated group. The degree of inhibition was reduced over time and by day 14, no inhibitory effect was observed in the 1 and 3 mg/kg-day groups, perhaps because of the upregulation of the NIS.

7.2.3.2 Effects on Thyroid and Pituitary Hormones and Thyroid Structure

Short-Term and Subchronic Studies in Rats and Mice

Fourteen-day studies (Caldwell et al. 1995; Springborn Laboratories Inc., 1998) and a 90-day study with a 30-day recovery period (Springborn Laboratories Inc., 1998) were conducted in rats to evaluate the effects of perchlorate on the thyroid function and structure and to see if these effects were reversible after discontinuation of exposure. The effects of perchlorate on the thyroid were also evaluated in 14- and 90-day studies in mice (Keil, et al., 1998; 1999) which were designed to investigate the immunotoxicity of perchlorate. Overall, these studies, demonstrated consistent associations between perchlorate exposure and effects on thyroid histopathology and morphometry as well as on pituitary and thyroid hormone levels. These results indicate a conservation in perchlorate sensitivity across species. The lowest reported LOAEL was 0.01 mg/kg-day, which was associated with changes in thyroid and pituitary hormone level in rats. The BMDLs identified for changes in thyroid histology in rats ranged from 0.008 to 2.09 mg/kg-day. The LOAEL for change in thyroid hormone concentrations in mice was 0.1 mg/kg-day which was the lowest dose tested. The LOAEL for colloid depletion, hypertrophy and hyperplasia in mice was 30 mg/kg-day. These studies are discussed in detail below.

Fourteen-Day Study in Rats (Caldwell et al., 1998)

Groups of Sprague-Dawley rats (six males and six females per group) were exposed to ammonium perchlorate in drinking water for 14 days at dose levels (male/female) ranging from 0.11/0.12, to 22.16/24.86 mg/kg-day (Caldwell et al., 1995; as cited in U.S. EPA, 2002). Endpoints examined included thyroid histopathology and morphometry, and pituitary and thyroid hormone levels.

Perchlorate exposure altered thyroid and pituitary hormone levels and thyroid histology. The lowest observed adverse effect levels (LOAELs) identified for changes in hormone levels for each group ranged from 0.11 - 0.44 (males) and 0.12 – 0.47 (females) mg/kg-day. The LOAEL identified for change in thyroid follicular lumen size was 1.11 (males) and 4.91 (females) mg/kg-day (Table 6).

Benchmark dose (BMD) analysis at 10% response level gave a lower limit of a one sided 95 percent confidence interval on the bench mark dose (BMDL) of 0.72 mg/kg-day for colloid depletion and 0.78 mg/kg-day for thyroidal cell hyperplasia (U.S. EPA, 2002). In the high dose groups, relative thyroid weights were significantly increased compared to the controls. These data demonstrated that short-term exposure altered thyroid histology, pituitary and thyroid hormone levels at low doses in rats.

Fourteen- and 90-Day Studies in Rats (Springborn Laboratories Inc., 1998)

Ammonium perchlorate was administered to male and female Sprague-Dawley rats (10 rats/sex/dose) at doses ranging from 0.01 to 10 mg/kg-day in drinking water for 14 and 90 days (Springborn Laboratories Inc., 1998; as cited in U.S.EPA 2002). An additional 10 rats/sex/dose at doses of 0.05, 1.0 and 10 mg/kg-day were sacrificed after a 30-day recovery period following cessation of the 90-day exposure to determine if the observed effects were reversible.

No other notable effects were observed in body and organ weights, food and water consumption, hematology, clinical chemistry, ophthalmology, gross necropsy, male and female reproductive parameters and histopathology of various tissues (the only effects observed were on thyroid function and structure).

Absolute thyroid weight and thyroid weight relative to both final body weight and brain weight were increased significantly in males at 10 mg/kg-day after 14 and 90 days of treatment and in females of the 10 mg/kg-day dose group after 90 days indicating LOAEL at 10 and a NOAEL at 1 mg/kg-day. Thyroid weights were returned to control values after the 30-day recovery period.

Table 6. Estimated NOAELS and LOAELS for Thyroid Hormone Changes in Rats

Species/Study	Time Point, (Doses, mg/kg-day)	Endpoint	Sex	NOAEL	LOAEL
Rat 14-Day (Caldwell et al., 1995)	14-Day (males – 0.0, 0.11, 0.44, 1.11, 2.26, 4.32, 11.44, 22.16) (females – 0.0, 0.12, 0.47, 1.23, 3.06, 4.91, 11.47, 24.86)	T3	M	0.11	0.44
			F	---	0.12
		T4	M	---	0.11
			F	---	0.12
		TSH	M	0.44	1.11
			F	---	0.12
		hTg	M	---	0.11
			F	---	0.12
		rT3	M	0.11	0.44
			F	0.12	0.47
		Colloid depletion	M/F combined		0.72 (BMDL)
		Thyroid hyperplasia	M/F		0.78 (BMDL)
		Thyroid hypertrophy			Not done
		Thyroid weight	M/F		11.44/11.47
		Thyroid Lumen size	M/F	0.44/0.47	1.11/4.91

Table modified and adopted from U.S.EPA (2002)

Thyroid histopathology was evaluated on days 14, 90, and 30 post exposure (120 days). Male rats appeared to be slightly more sensitive, exhibiting follicular cell hyperplasia by day 14 and

not recovering fully for any of the thyroid histopathological indices by 30 days post exposure. On day 14, females showed decreased colloid and follicular cell hypertrophy at 10 mg/kg-day. Males also showed a significant increase in these two thyroid response measures at this dose but also exhibited changes at lower doses and in addition showed hyperplasia. By 90 days, all three response measures (colloid depletion, follicular cell hypertrophy, and follicular cell hyperplasia) in both sexes were significant at 10.0 mg/kg-day, again indicating a LOAEL at 10 and a NOAEL at 1 mg/kg-day. Recovery of the thyroid histopathological changes was essentially complete by 30 days post exposure although the males did have some indication of residual toxicity. The BMD analyses for these data are found in Table 7. Data for females and males were combined. The BMDLs for colloid depletion and hypertrophy at 14 days were 0.28 and 0.017 mg/kg-day, respectively with no estimate for hyperplasia. By 90 days, the BMDL values decreased for colloid depletion and hypertrophy to 0.03 and 0.008 mg/kg-day. The BMDL value for hyperplasia was 2.09 mg/kg-day (Table 7).

U.S. EPA (2002a) also analyzed the thyroid hormone data using two-way ANOVA tests, one for each of the three hormones, to allow for a statistical comparison of the interaction between gender, time, and treatment. In general, the analysis demonstrated only a marginal interaction between gender and treatment, resulting from a slight difference in magnitude of effects between genders. However, no differences in LOAELs between genders were observed (with minor exceptions likely caused by small changes in variance between groups, which are probably not biologically significant).

There were significant day-by-gender-by-treatment interactions for T3 on day 14 and day 90. The NOAEL for T3 in females was 10 mg/kg-day. The low potency of perchlorate on T3 in females at the 14 day time point may be artifactual. U.S.EPA (2002) concluded that the group mean for females for the 14 day time point may be artificially low relative to some of the other studies. Thus, the biological significance of this gender-dependent effect of perchlorate after 14 days of exposure is suspect. Consistent with this conclusion is the significant dose-dependent decrease in T3 concentrations in female rats exposed to 0.12 to 25 mg/kg-day perchlorate in a previous 14 day exposure study by Caldwell et al., 1995. The LOAEL for effects on T3 for both males and females was 0.01 mg/kg on day 90.

The NOAEL for effects on T3 at day 120 was 1.0 mg/kg-day, indicative of a recovery of T3 concentrations after cessation of treatment. There were significant day-by-treatment interactions for effects on T4 at the 90- and 120-day time points but not at the 14-day time point. For day 14 data, a LOAEL of 0.05 mg/kg-day was identified for effects on T4. A LOAEL of 0.01 mg/kg-day was identified for effects on T4 in both sexes on day 90. Analysis of the data from the 30-day recovery period (the day 120 time point) revealed a LOAEL of 0.05 mg/kg-day in males and a NOAEL of 1.0 mg/kg-day in females for effects on T4. Perchlorate caused a dose-dependent increase in TSH that was apparent at the day 14 and day 90 time points. However, the effect does not seem to be duration dependent. The NOAEL for effects on TSH at day 14 was 0.01 mg/kg-day in the males. The 0.01 mg/kg-day dose was a LOAEL in the females. This small difference between males and females is likely caused by small changes in variance between groups rather than by a biologically significant difference. The TSH concentrations did not recover to control values 30 days after cessation of treatment with a LOAEL at 0.05 mg/kg-day in both sexes. The data demonstrate a dose- and time-dependent effect of perchlorate on thyroid hormones and

TSH. There was no NOAEL established in this data set due to multiple effects at the lowest dose of 0.01 mg/kg-day. There was some evidence of recovery at the day 120-evaluation (30 days after cessation of treatment). The NOAEL for effects on T3 increased to 1.0 mg/kg-day. However, the omission of the 0.01 mg/kg-day dose group at the 120-day time point makes it difficult to make any conclusion about the recovery of effects on T4 and TSH (Table 7).

Table 7. LOAELS and NOAELS Determined for Changes in Pituitary and Thyroid Hormones

Species/Study	Time Point, Age (Doses, mg/kg-day)	Endpoint	Sex	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Rat 14-Day (Springborn Laboratories Inc., 1998))	14-day (0, 0.01, 0.05, 0.2, 1.0, 10)	T3	M	ND ^a	0.01
			F	10	---
		T4	M	---	0.05
			F		
		TSH	M	0.01	0.05
			F	---	0.01
		Thyroid weight	M	1	10
			F	---	---
		Colloid depletion	M+F		0.28
		Hypertrophy	M+F		0.017
		Hyperplasia			Not estimated
	90-day (0, 0.01, 0.05, 0.2, 1.0, 10)	T3	M	ND	0.01
			F	ND	0.01
		T4	M	ND	0.01
			F	ND	0.01
		TSH	M	0.05	0.2
			F		
		Thyroid weight	M	1	10
			F	1	10
		Colloid depletion	M+F		0.03 (BMDL)
		Hypertrophy	M+F		0.008 (BMDL)
		Hyperplasia	M+F		2.09 (BMDL)
Rat subchronic study (Springborn Laboratories Inc., 1998)	120-day (90 day exposure, 30 day recovery) (0, 0.05, 0.21.0, 10)				
		T3	M		
			F	1.0	10
		T4	M	ND	0.05
			F	1.0	10
		TSH	M	ND	0.05
			F	ND	0.05

Table modified and adopted from USEPA (2002)

^aND = not determined

Fourteen- and 90-Day Studies in Mice (Keil et al., 1998; 1999)

In studies designed to investigate the immunotoxicity of perchlorate, mice were exposed to perchlorate doses ranging from 0.1 to 30 mg/kg-day for 14 or 90 days (Keil et al., 1998; 1999; as cited in U.S.EPA, 2002). Increased thyroid colloid depletion, hypertrophy and hyperplasia were observed at 30 mg/kg-day dose levels in these studies. At 30 mg/kg-day perchlorate dose level, intrafollicular capillaries were congested and the nuclear to cytoplasmic ratio of the follicular cells was altered.

In the hormone analysis, mean contrast testing showed a LOAEL of 0.1 mg/kg-day for T3. However, the dose-related decrease in T3 was not linear in that the T3 levels in the 0.1 and 0.3 mg/kg-day dose groups differed from controls but the T3 levels in the 1 and 3.0 mg/kg-day dose groups did not. T4 was not altered after 14 days of treatment with a NOAEL of 30 mg/kg-day; whereas, after 90 days of treatment, the LOAEL was 0.1 mg/kg-day. There was no change in TSH levels, contrary to changes in thyroid histopathology discussed previously.

7.2.4 Developmental Neurotoxicity Studies

Since iodine deficiency in pregnant subjects had been demonstrated to alter thyroid and pituitary hormone levels resulting in neuropsychological deficit in offspring, various developmental/reproductive studies were conducted to determine if perchlorate exposure could also cause the same effects observed in iodine deficient mothers and offspring based on its proposed mode of action.

Three developmental neurotoxicity studies [Argus Research Laboratories, Inc. (1998a); Bekkedal et al. (2000); Argus Research Laboratories, Inc. (2001)] were conducted to investigate the effects of perchlorate on developing rat brain structure and behavior. In the first neurodevelopmental study conducted by Argus Research Laboratories, Inc. (1998a), the most notable effects of perchlorate treatment were observed in the F1-generation pups. The effects included: (a) brain morphometric changes, especially increase in the size of the corpus callosum in the 10 mg/kg-day dose group, and possible changes in other brain regions in the 3 mg/kg-day dose group; (b) thyroid hormone (T3, T4) decreases in the 0.1 and 1.0 mg/kg-day dose group, and an accompanying pituitary hormone (TSH) increase in the 3 mg/kg-day dose group; and, (c) increase in motor activity in some age groups. Evaluation of the thyroid histopathology data indicated that pups were also the most sensitive with a BMDL ranging between 0.009 and 0.33 mg/kg-day for increases in colloid depletion.

In response to recommendations at the 1999 peer review of the U.S.EPA (1998) document, the United States Navy (USN) (Bekkedal et al. 2000) performed a neurodevelopmental study that included evaluation of motor activity. By combining the Argus Research Laboratories, Inc. (1998a) and the Bekkedal et al. (2000) studies, and using Bayesian analysis, U.S.EPA (2002) determined a NOAEL of 1.0 mg/kg-day for changes in motor activity.

Because of the effects observed in the Argus Research Laboratories, Inc. (1998) study on brain morphometry, an additional neurodevelopmental study was conducted by the same laboratory in 2001. In the neurodevelopmental study conducted by Argus Research Laboratories, Inc. (2001),

the LOAEL identified for changes in brain morphometry in pups exposed to perchlorate *in utero* and after birth was 0.01 mg/kg-day. The LOAEL for changes in T4 and TSH levels in maternal animals on the last day of pregnancy was also 0.01 mg/kg-day, suggesting that the mothers were hypothyroid during pregnancy which may have initiated altered brain structure in the pups. In postnatal day 21 (PND21) pups, the LOAEL for changes in ammonium perchlorate-induced T4 levels was 0.01 mg/kg-day. The BMDLs calculated for decreases in T4 in maternal animals and pups were 0.004 mg/kg-day and 2.86×10^{-7} mg/kg-day, respectively. Changes in thyroid histology were also observed in both the dams (BMDLs ranging between 0.13 and 8.51 mg/kg-day ammonium perchlorate doses) and their pups (BMDLs ranging between 0.04 and 2.17 mg/kg-day). These studies are discussed in detail below.

Developmental Neurotoxicity Study (Argus Research Laboratories, Inc., 1998a)

Female rats (25/dosage group) were administered target doses of 0, 0.1, 1.0, 3.0, and 10 mg/kg-day perchlorate in the drinking water beginning on gestation day zero (GD0) and ending at scheduled sacrifice. Thyroids from dams were weighed and evaluated histologically. Five dams per group were selected for sacrifice at PND10. Thyroid and pituitary hormone analyses (T3, T4, TSH) were performed on blood. Post-weaning pups that were not selected for continued observation were sacrificed and necropsied on PND5. Blood was sampled for thyroid and pituitary hormone analysis, and thyroids were examined histologically. F1-generation pups that were not selected for continued observation on PND10 were sacrificed and examined for gross lesions. Post-weaning pups that were selected for continued observation were given ammonium perchlorate in the drinking water and were assigned to four different subsets for perchlorate toxicity evaluations:

- (1) The first male and female group of pups (1/sex/dose; total of 97 male and 100 female pups) were assigned randomly to Subset 1 for brain weight and neurohistological examination (including morphometric measurements). All pups were selected for fixed brain weights on ¹²(PND12); 6/sex/dose (total of 30 male and 30 female pups) were selected for neurohistological examination.
- (2) The second male and female group of pups (1/sex/dose; total of 100 male and 100 female pups) were assigned randomly to Subset 2 for passive avoidance testing on PNDs 23 to 25 and PNDs 30 to 32; water maze testing on PNDs 59 to 63 and PNDs 66 to 70; and scheduled sacrifice at PNDs 90 to 92, with blood collection for thyroid and pituitary hormone analysis.
- (3) The third male and female group of pups (1/sex/dose; total of 100 male and 100 female pups) were assigned randomly to Subset 3 for motor activity evaluation on PNDs 14, 18, 22, and 59; auditory startle habituation on PNDs 23 and 60; and scheduled sacrifice on PNDs 67 to 69.
- (4) The fourth male and female group of pups (1/sex/dose; total of 100 male and 100 female pups) were assigned randomly to Subset 4 for regional brain weight evaluation on PNDs 81 to 86 (6/sex/dose; total of 30 male and 30 female rats) and neurohistological on PNDs 82 to 85 (6/sex/dose; total of 30 male and 30 female rats).

² It should be noted that Argus Laboratories identifies the day of birth as PND1; for example the day PND 5 and PND10 actually correspond to PND4 and PND9 in this study. The above description of the study design use the Argus nomenclature.

The results of the neurodevelopmental study reported by Argus Research Laboratories, Inc. (1998a; as cited in U.S. EPA, 2002) are discussed below.

No treatment-related effects on food or water consumption, mortality, clinical signs, necropsy, body weight, or pregnancy outcome measures (Argus Research Laboratories, Inc., 1998a) were observed in the F0-generation dams.

Results of general toxicity in the pups (F1-generation) revealed no treatment-related effects on feed consumption, mortality, clinical signs, body weight, or sexual development. No treatment-related effects were observed on mortality, brain weight, or body weight in the pups of Subset 1 at PND12, Subset 2 at PNDs 90 to 92, or Subset 3 at PNDs 67 to 69.

In the Subset 1 subgroup subjected to neurohistological examination (the F1 pups were sacrificed on PND12), morphometric analyses revealed a 23.4% increase in the size of the corpus callosum in females and a 30.2% increase in males (not significant) at the high dose (10 mg/kg-day). Slight decreases in brain weight were also noted at the highest dose in females. In Subset 4 (the F1 pups sacrificed on PND82), there was a continued effect on the size of the corpus callosum (20.9% increase) in males, but no effect in females at the highest dose. There was also a 3.4% increase in the brain weight in males and increases in the size of the frontal cortex (9.2%) and the caudate putamen (10.2%).

The EPA concluded that the effects on the brain regions may be significant and that analyses of the next lower dose were warranted which were requested from the sponsor (PSG). The additional analysis on brain morphometry was performed at the next lower dose of ammonium perchlorate (3.0 mg/kg-day) in the Subset 1 pups at PND12. In addition to previous findings, the new analysis showed a statistically significant increase in the anterior/posterior cerebellum size, a statistically significant decrease in the caudate putamen for the PND12 female pups, and a statistical significant decrease in the hippocampal gyrus size for the PND12 male pups. These effects were not considered treatment-related by the Primedica/Argus pathologist because they were not dose dependent.

A preliminary reanalysis by EPA of the control, 3- and 10-mg/kg-day groups was restricted to the corpus callosum because this was the area with the largest effect (U.S EPA, 2002). There was a significant increase in the size of the corpus callosum only in the 10-mg/kg-day group and no interaction of gender and treatment was observed. EPA did not agree with the Argus Research Laboratories, Inc. (1998a) argument that these effects were “not suggestive of a neurotoxic effect” because of “an unknown biological significance.” U.S.EPA considered the changes observed in various regions of the brain to be adverse effects, and determined 10 mg/kg-day dose level as the LOAEL and 3 mg/kg-day dose level as a NOAEL for these changes in brain histology.

Additional analyses of the brain morphometry were provided by the U.S.EPA at the 1999 external peer review (U.S.EPA, 2002) that corroborated the preliminary U.S EPA analysis. The data were analyzed using a 2-way ANOVA, with dose and sex as independent variables. Significant effects of dose were found in corpus callosum, hippocampal gyrus, anterior and posterior cerebellum, and caudate putamen. An effect of sex was also found in caudate putamen.

The effect on corpus callosum was confirmed and showed an increase in size at the 10 mg/kg-day dose. Hippocampal gyrus (12% less than control) and caudate putamen (7.3% less than control) showed a decrease in size at the 3 mg/kg-day dose, with no significant difference between control and high dose, yielding a U-shaped dose response. The anterior and posterior cerebellum showed a significant increase in size at the 3 mg/kg-day group (13%).

In the thyroid histopathology investigations, the F0-generation dams exhibited colloid depletion, hypertrophy and hyperplasia. A clear dose-response was not evident, however, with the possible exception of colloid depletion at levels above 0.1 mg/kg-day. Colloid depletion and hypertrophy were observed in PND4 pups at 0.1 and 3 mg/kg-day dose level. There was a significant effect of perchlorate treatment on thyroid lumen size for all doses at PND5 with a LOAEL of 0.1 mg/kg-day. Colloid depletion and increases in hypertrophy were observed in PND4 pups at 0.1 and 3 mg/kg-day dose levels. U.S. EPA (2002) performed benchmark dose analysis and found BMDL estimates for colloid depletion, hypertrophy, and hyperplasia of 0.33, 0.88, and 3.62 mg/kg-day in PND4 pups when using a model where the exponent on dose is restricted to be ≥ 1 . When the mathematical model was not restricted, the BMDL obtained for colloid depletion was 0.009 mg/kg-day. Histopathology in the animals from PND90 and PND92 indicated variable effects on colloid depletion, hypertrophy, and hyperplasia. A BMDL was calculated with confidence for colloid depletion with a resultant estimate of 0.03 mg/kg-day. There was no significant effect perchlorate treatment on lumen size in PND90 pups. In summary, evaluation of the thyroid histopathology data indicated that pups are the most sensitive with a BMDL between 0.009 and 0.33 mg/kg-day for increases in colloid depletion.

Thyroid and pituitary hormone levels were also significantly altered due to perchlorate treatment. T4 and T3 levels were significantly decreased with accompanying increase in TSH levels in PND5 pups. The NOAELs identified in PND5 pups for changes in T3, T4 and TSH levels were 0.1, 0.1 and 3 mg/kg-day ammonium perchlorate doses, respectively.

Behavioral evaluation was performed on Subset 3 pups. The behavioral study showed a dose-dependent increase in motor activity in male rats at PND14, but the changes observed in motor activity were not statistically significant. No other perchlorate-induced changes were detected in any other behavioral indices (i.e., passive avoidance, water maze auditory startle). There were also no changes detected in motor activity at any other ages (i.e., PND18, PND22, PND59). The U.S. EPA questioned why the method or statistics did not detect significance for the dose-dependent increase in total session counts that amounted to a 95% increase over controls in the highest dose groups. Even though the increase in motor activity was not statistically significant, U.S. EPA (2002) maintained that this effect should be considered biologically significant and determined BMDLs of 1.04 and 0.66 for number of movements and time spent in movement, respectively. U.S. EPA and suggested that these BMDLs could serve as estimates of NOAELs for this data set.

As indicated previously, because of concerns on the effects of perchlorate on motor activity in treated rats, an additional neurotoxicity study was recommended by the 1999 external peer review on the U.S. EPA (1998) document. In response, the United States Navy (USN) performed a study that included evaluation of motor activity in rats of both sexes (Bekkedal et al., 2000; as cited in U.S. EPA, 2002).

Motor Activity Study (Bekkedal et al., 2000)

Female Sprague-Dawley rats were dosed with ammonium perchlorate for two weeks at 0, 0.1, 1.0, 3.0 or 10.0 mg/kg-day prior to mating with the breeder males and through PND10. On PND14, one male and one female were randomly selected from each litter to be used in the motor activity testing. These same animals were tested on PND14, PND18 and PND22. Nine different measures of motor activity were automatically recorded using Opto-Varimex activity meters at ten-minute intervals. The measures included: frequency and time of ambulatory movements, frequency and time of stereotypic movements, frequency of movements in the horizontal plane, distance traveled in the horizontal plane, frequency of rears, total number of horizontal movements made while in the rearing position (vertical plane movements), and time spent resting.

Bekkedal et al. (2000; as cited in U.S. EPA, 2002) found no statistically significant difference for the main effect of perchlorate exposure for any of the 9 measures nor any reliable interaction related to dose. The authors, however noted that there was a general pattern of dose-dependent changes in the latter session (90-minute). They also noted that this pattern, as in the previous Argus Research Laboratories, Inc. (1998a) data, suggested that exposed pups had slightly slower rate of habituation and thus maintained a higher level of activity as compared to untreated rats.

From the results of the Bekkedal et al. (2002) study, U.S. EPA noted that a similar pattern of effects seen in the Argus Research Laboratories, Inc. (1998a) study was again emerging. As a result, EPA requested that NIEHS perform a statistical analysis that could formally integrate the various measures together, as well as statistically compare the two studies with each other. After rigorous statistical analysis of both the Argus Research Laboratories, Inc. (1998a) and the Bekkedal et al. (2000) (the U.S Navy study) studies using Bayesian analysis, U.S.EPA (2002) determined a NOAEL of 1 mg/kg-day for effects of perchlorate on motor activity of pups exposed *in utero* and postnatally.

*Developmental Neurotoxicity Study (Argus Research Laboratories, Inc., 2001)**Brain Morphometry Changes*

The Argus Research Laboratories, Inc. (2001) study was conducted in response to recommendations made at the 1999 external peer review of the U.S.EPA (1998) document for additional analyses of thyroidal and brain effects during gestation and post-natal days (U.S.EPA, (2002).

Female Sprague-Dawley rats were treated with ammonium perchlorate at 0, 0.01, 0.1, 1.0, or 30 mg/kg-day in drinking water two weeks prior to cohabitation and continuing through the day of sacrifice. F1-generation pups were not directly dosed but might have been exposed *in utero* during gestation and via maternal milk and maternal water during the postpartum period. Thyroid and brain histological and morphometric evaluations were conducted on PND4, PND9, and PND21 pups. Thyroid histopathology analysis was also performed in dams at various time points.

Various types of statistical analyses were performed on the Argus Research Laboratories, Inc. (2001) data both by Argus Laboratories Inc. (2001) and U.S.EPA (2002). In the Argus Research Laboratories, Inc. report (2001; as cited in U.S.EPA, 2002), statistical analysis consisted of Students t-test comparisons between controls and the corresponding group of each sex at each separate dose level. The Argus Laboratories Inc. (2001) analysis found a large number of significant effects in brain morphometry for both PND9 and PND21 time points in rat pups that were exposed to ammonium perchlorate during gestation and postnatally (U.S. EPA, 2002).

In the PND9 pups, the Argus Laboratories, Inc. (2001; as cited in U.S.EPA, 2002) analysis found no significant effects of treatment or sex on brain weight, anterior-posterior cerebrum length, or anterior-posterior cerebellar size. Significant increases in sizes of frontal, parietal and striatum (male rats) were observed at 1.0 mg/kg-day doses (Table 8). There was a decrease in the size of the striatum at 0.1 mg/kg-day dose level and decrease in the size of region CA1 of females at the 0.01, 0.1 and 1.0 mg/kg-day dose levels. In the PND 21 pups, the Argus Laboratories Inc. (2001) report found significant decreases in size of the striatum at 0.01, 0.1, and 1.0 mg/kg doses and increases in the size of the corpus callosum (posterior) and cerebellum at the same doses. Decrease in the size of CA3 at the 0.1 mg/kg-day dose, decrease in anterior corpus callosum in females at 0.01 mg/kg-day, and increase in the size of the frontal region in males at 0.1 and 30 mg/kg-day doses were also observed.

While the analysis in the Argus report demonstrated significant treatment-related effects, the number of t-test run increases the risk of Type I error into the analysis. To address this issue, U.S.EPA (2002) used a multivariate analysis, profile analysis. Profile analysis is more conservative than the Students' t-test analysis because a multiple analysis of variance (MANOVA) takes into account any correlation between the independent variables, whereas, the multiple t-tests assume complete independence. The profile analysis also reduced the number of main effects by nesting gender within litter and by constructing a vector composed of all of the morphometric measurements from each animal, then comparing these vectors. Treatment effects within brain region were examined with univariate analysis of variance with gender nested within litter.

The profile analysis showed significant exposure related changes in relative growth of different brain regions, even at the lowest administered doses. Univariate analysis was used to further investigate differences within brain regions. In PND9 pups, the sizes of the frontal and parietal regions of the cerebral cortex and the striatum were significantly increased at 1.0 mg/kg-day ammonium perchlorate dose. The size of the corpus callosum was increased at 0.1 mg/kg-day dose only in males. In female pups the size of the striatum was decreased at 0.1 mg/kg-day and the size of the CA1 region of the hippocampus was decreased at 0.01 mg/kg-day dose level. While most changes in the investigated regions are in the range of ± 5 to 11%, the increase in size in the corpus callosum was 23%. Harry (2001; cited in U.S EPA 2002) reported that a post-hoc analysis of the plane of cut of the PND9 brain sections suggested that the 0.1 and 1.0 mg/kg-day dose groups were sectioned at different depths than were the other dose groups. This likely contributed to the small but significant increase in the size of the frontal, parietal, and striatal sections in the 1.0 mg/kg-day dose groups and may have contributed to the large increase in the size of the anterior corpus callosum seen in the PND9 males. Also, many of the PND9 brains showed signs of damage that may have compromised the measurements.

The profile analysis of the PND9 brain morphometric results (U.S.EPA, 2002) were similar to those obtained by Argus Laboratories Inc. (2001) using Students t-test except for CA1 region where the Argus Laboratories Inc. (2001) analysis did not detect a significant difference in female CA1 at the 0.01 mg/kg-day dose.

In the PND21 brain morphometric data, where sectioning problems were not a concern, significant differences in the parallel-profile test demonstrated exposure related changes in relative growth of different brain regions even at the lowest administered doses (Figure 5). Univariate analysis was used to further investigate differences within brain regions. Significant alterations in the striatum, cerebellum and corpus callosum were observed at 0.01 mg/kg-day (Table 8). The size of the striatum was significantly reduced at all doses except the highest dose (30 mg/kg-day) giving a U-shaped dose-response curve. The posterior regions of the corpus callosum increased in size (24% increase in size at 0.01 and 0.1 mg/kg-day and 39% increase at 0.1 mg/kg-day) with dose in an inverted U-shape. U.S.EPA (2002) concluded that inverted U-shaped or U-shaped dose-response curves are not uncommon in biological systems as compensatory or other mechanisms may be triggered at high doses. In female pups, decreases in the CA3 region of the hippocampus (at 0.1 mg/kg-day) and the anterior corpus callosum (at 0.01 mg/kg-day) were observed while in male pups, increase in the frontal region of the cerebral cortex was observed at 0.1 mg/kg-day.

Although the PND9 brain morphometric measurements corroborated the PND21 data, U.S. EPA (2002) relied on the PND21 measurements in its weight of the evidence evaluation for perchlorate toxicity because of the concerns raised by Harry (2001) on the depth of the sectioning of the brain regions. ORS concurs with the above U.S. EPA decision and similarly used the PND21 data in its evaluation of weight of the evidence to derive an RfD for perchlorate.

Table 8. Results in Brain Morphometry Changes Observed in PND9 and PND21 Rat Pups

Brain region	PND9 LOAEL (mg/kg-day)	PND21 LOAEL (mg/kg-day)	Remarks
Corpus Callosum (posterior)	↑0.1 (^b m)	↑0.01	While most of the changes in the various brain regions were ±5 to 11%, the male corpus callosum was increased 23% at both 0.1 and 1 mg/kg-day dose in PND9 pups, and in the PND21 pups the change in the size of corpus callosum was increased in size 24% at 0.01 and 1.0 mg/kg-day dose groups and 39% in the 0.1 mg/kg-day dose group (U.S.EPA, 2002)
Corpus Callosum (anterior)	ND ^a	↓0.01 (f)	
Cerebellum	ND	↑0.01	
Striatum	↑ ^d 1.0 (m) ↓0.1(^c f)	↓0.01	
Frontal cortex	↑1.0	↑0.1	
Parietal	↑1.0	ND	
CA1 region of hippocampus	↓ ^d 1.0 ↓0.01 (f)	ND	
CA3 region of hippocampus		↓0.1	
External germinal	↓1.0	ND	

^aND = no data

^bm = male

^cf = female

^d↑ = increase in size, ↓ = decrease in size

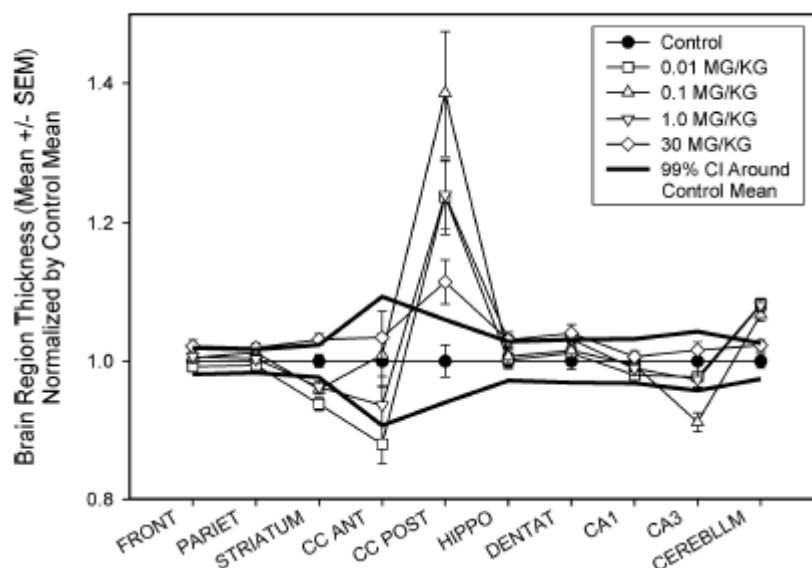


Figure 5. Profile Analysis of Brain Morphometry Measurements For PND21 Rat Pup Brain Regions. The male and female data on linear thickness measurements were combined and normalized by the control mean of each region. The control data are represented by the horizontal line at 1.0. Profile analysis determines whether the vectors of measurements from each treatment group differ from each other and control in a dose-dependent fashion. The heavy line represents the $\pm 99\%$ confidence interval around the mean control values. Note that while this plot uses the normalized data to more easily illustrate the data vectors, the actual analysis was performed using raw data values (adopted from U.S.EPA, 2002).

In summary, exposure to perchlorate in drinking water at low doses during conception, embryo-fetal development and lactation produced significant morphometric changes in newborns in various regions of the brain, especially the corpus callosum, where a 23% increase in size was observed at 0.01 mg/kg-day. ORS considers alterations in brain regions of animals to be an adverse effect and determined a LOAEL of 0.01 mg/kg-day ammonium perchlorate for these effects. This LOAEL was also the basis for the U.S.EPA (2002) updated draft RfD.

Following completion of the initial draft of this report, unpublished issues regarding the histopathological analyses of the brain regions performed in the Argus (2001) study were raised. U.S. EPA (2003) has performed a reanalysis of these data, and results from this effort are now available. The reanalysis of the brain morphometric measurements for the corpus callosum and the striatum in the U.S. EPA (2003) report are consistent with the measurements presented in the U.S.EPA (2002) document. ORS believes it is prudent to continue to include this data in the weight of the evidence analysis it is using to derive an RfD for perchlorate. The reasons for including the brain morphometric assessments in the weight of the evidence evaluation include: 1) the data on the effects in the corpus callosum were statistically significant and consistently observed in two separate studies; 2) Statistically significant effects were also reported for the cerebellum and striatum; and, 3) effects on brain morphometry are mechanistically consistent with the observed disruption of thyroid hormone status that was observed at the same dose levels. This weight of the evidence approach includes data on brain morphometry effects as well as other endpoints.

Changes in Thyroid Function and Structure

Dams examined at various time points had significantly increased colloid depletion, thyroid cell hypertrophy and hyperplasia. Benchmark dose analysis of the thyroid histopathology data from dams investigated at various time points (from GD21 through PND21) gave BMDL values ranging from 0.13 to 1.01, 1.01 to 1.24, and 0.92 to 8.51 for colloid depletion, hypertrophy and hyperplasia respectively. The effects of ammonium perchlorate on the thyroids of pups were largely limited to colloid depletion. The BMDLs for colloid depletion from GD21 – PND21 male and female pups combined ranged from 0.12 - 2.17 mg/kg-day. For GD21 – PND21 male pups the values ranged from 0.12 – 1.36 mg/kg-day and for GD21 – PND21 female pups, the range was from 0.04 - 1.24 mg/kg-day. Hyperplasia was also observed in PND21 pups at 13.7 mg/kg-day for male and female pups combined, and at 5.45 mg/kg-day for male pups (Table 9). No hyperplasia was observed in female pups. The BMDL increases with age in the pups suggesting that the thyroid gland may be most susceptible to the effects of perchlorate during gestation or at the time of parturition. This may be because of perturbation of pup thyroid function and lack of protection of the pup by the mother because of the compromised function of her own thyroid (U.S. EPA, 2002).

Table 9. Benchmark Dose Lower Confidence Limit Estimates from Thyroid Histopathology from the Argus Research Laboratories, Inc. (2001)

Age	Colloid Depletion	Hypertrophy	Hyperplasia
	BMDL (mg/kg-day)	BMDL (mg/kg-day)	BMDL (mg/kg-day)
GD21-PND21 Dams	0.13-1.01	1.01-1.24	0.92-8.51
GD21-PND21 Pups (m)	0.12-1.36	NOE ^a	NOE
GD21 – PND21 pups (f)	0.04-1.24	NOE	NOE
GD21 – PND21 pups (m+f)	0.12-2.17	NOE	NOE
PND21 (m)			13.7
PND21 (m+f)			5.45

Table modified and adopted from U.S. EPA (2002a)

^aNOE= No observed effect

Pituitary (TSH) and thyroid (T3 and T4) hormones were also significantly affected by perchlorate treatment in both dams and their fetuses and offspring, leaving the dams and offspring in a hypothyroidic state. T4 was significantly decreased in the dams on GD21, PND9, and PND21. The LOAELs for T4 ranged between 0.01 and 30 mg/kg-d. The LOAEL increased with age in dams, the lowest (0.01 mg/kg-day) being observed at GD21. A BMDL was only calculable for T4 at GD21 and was 0.004 mg/kg-day. The LOAEL on PND9 for T3 was 30 mg/kg-day. The LOAELs for TSH in dams were 0.01, 0.01, and 0.1 mg/kg-day on GD21, PND9 and PND21 respectively (Table 10). Benchmark dose analysis resulted in a BMDL of 0.53 mg/kg-day for TSH on PND21.

In fetuses and offspring, T3 and T4 levels were evaluated at PND4, PND9, and PND21. The LOAEL for T3 at PND4 was 0.01 and at PND21 the LOAEL was 1.0 mg/kg-day. A BMDL was calculable only for the male pups at PND21 with a resultant value of 0.13 mg/kg-day. T4 levels were significantly decreased compared to controls at GD21, PND4 and PND9 and the LOAEL was 0.1 at all time points. At PND21, males appeared to be more sensitive than females since the LOAELs for the reduction of T4 in males and females were 0.01 and 0.1 mg/kg-day respectively. The BMDL estimate for T4 at PND21 was very low and resulted in a value of 2.86

$\times 10^{-7}$ mg/kg-day. TSH was significantly increased on GD21 and PND9 with LOAELs of 1 and 0.01 mg/kg-day respectively. At PND21, males appeared to be more sensitive for effects on TSH. The LOAEL for males was 0.01 mg/kg-day while for females it was 0.1 mg/kg-day. Benchmark dose analysis on combined data resulted in a BMDL of 0.02 mg/kg-day for TSH (Table 10).

Table 10. LOAELs and BMDLs for Effects on Thyroid and Pituitary Hormones (Argus Research Laboratories Inc., 2001)

Sprague-Dawley rats		T3 Concentration		T4 Concentration		TSH Concentration	
Age	Generation	LOAEL (mg/kg-day)	BMDL (mg/kg-day)	LOAEL (mg/kg-day)	BMDL (mg/kg-day)	LOAEL (mg/kg-day)	BMDL (mg/kg-day)
GD21 ^a	dams	30	NC ^b	0.01	0.004	0.01	NC
PND9	dams	30	ND ^c	1	ND	0.01	
PND21	dams	30	NC	30	NC	0.1	0.53
GD21	fetus	0.01		0.1		1.0	
PND4	pups	0.01	NC	0.1		NE ^d	
PND9	pups	0.01		0.1		0.01	
PND21	pups	1	0.13 (m)	0.01 (m; NE in f)	2.86 $\times 10^{-7}$ (m) 0.001 (BMD)	0.01 (m) ^e 0.1 (f) ^f	0.02 (m +f) 0.06 (BMD)

Table modified and adopted from U.S. EPA, 2002

^aGestation and postnatal days are reported according to U.S.EPA designation

^bNC = not calculable

^cND = not determined

^dNE = no significant effect

^em = male

^ff = female

7.2.5 Developmental and Reproductive Toxicity Studies

Developmental toxicity studies of perchlorate were performed in rabbits (Argus Research Laboratories, Inc., 1998b) and rats (Argus Research Laboratories, Inc., 2000) as testing guidance for developmental toxicity requires data in two different species (U.S.EPA, 2002). In rabbits, the LOAEL for decrease in T4 levels was 1 mg/kg-day and the BMDL for changes in colloid depletion and hyperplasia were 0.008 mg/kg-day and 0.42 mg/kg-day respectively. The NOAEL identified for fetotoxicity other than that which may have occurred in the thyroid was >100 mg/kg-day. In rats, the LOAEL identified for maternal and developmental effects was 30 mg/kg-day and the NOAEL was 3 mg/kg-day. These studies are discussed below.

Developmental Toxicity Study in Rabbits (Argus Research Laboratories, Inc., 1998b)

New Zealand white rabbits were treated with 0, 0.1, 1.0, 10, 30 and 100 mg/kg-day ammonium perchlorate in drinking water from GD6 to GD28 (Argus Research Laboratories, Inc., 1998b; as cited in U.S.EPA, 2002). Rabbits were sacrificed on GD29. The only remarkable histopathology in the maternal animals was observed in the thyroid. There were significant increases in colloid depletion, hypertrophy and hyperplasia at 1.0 mg/kg-day. Benchmark dose analysis resulted in BMDLs of 0.008 mg/kg-day for colloid depletion and 0.42 mg/kg-day for hyperplasia. No BMDL could be calculated for hypertrophy because of poor model fit. No fetal developmental

toxicity was observed even at the highest dose. The fetal NOAEL identified for embryo-fetal developmental toxicity was greater than 100 mg/kg-day. It appears from the data that fetal thyroid structure and function were not investigated.

Maternal thyroid and pituitary hormone analysis demonstrated that T3 and TSH levels were not significantly altered at any dose level while T4 levels were significantly decreased compared to controls starting at 1.0 mg/kg-day. Thus, the LOAEL for change in T4 was 1.0 mg/kg-day and the NOAEL was 0.1 mg/kg-day. The lack of effect on T3 and TSH is contrary to what is observed in rats.

Developmental Toxicity Study in Rats (Argus Research Laboratories, Inc., 2000)

Rats (24/dose group) were treated with 0, 0.01, 0.1, 1.0 or 30 mg/kg-day ammonium perchlorate in drinking water starting two weeks before cohabitation and continuing until the day of sacrifice (Argus Research Laboratories, Inc., 2000). Three dams in the 30 mg/kg-day group showed significantly increased localized alopecia which U.S.EPA (2002) felt should be considered treatment-related and biologically significant. In agreement with U.S.EPA (2002), this report also considers alopecia to be treatment related. Sparsity of hair growth in marmoset (Mano et al., 1987) and absence of wool growth in sheep (Potter et al., 1981) fed iodine-deficient diets have been observed. Alopecia appears to be not a direct effect of perchlorate, but may be due to perchlorate's interference with iodine uptake. No other treatment-related maternal effects were observed. Preimplantation losses of 12%, 18%, 20%, 16% and 25% were observed in the 0, 0.01, 0.1, 1.0, and 30 mg/kg-day groups respectively. U.S.EPA (2002) stated that the biological significance of these effects was unclear, while CA EPA (2002) found using statistical analysis that the increase in preimplantation loss was statistically significant at the 30 mg/kg-day dose level. The number of live fetuses and ossification per litter for sternal centers and phalanges were significantly reduced at 30 mg/kg-day. The LOAEL identified for maternal and developmental effects was 30 mg/kg-day and the NOAEL was 3 mg/kg-day.

Two-Generation Reproductive Study (Argus Research Laboratories, Inc., 1999)

A two-generation reproductive study was conducted to characterize the potential toxicity of perchlorate on reproductive parameters. The BMDL across generations ranged from 0.11 to 0.9 mg/kg-day for colloid depletion, from 0.057 and 0.32 mg/kg-day for thyroid hypertrophy, and from 0.0004 to 2.44 mg/kg-day for thyroid hyperplasia. Changes in T4 and TSH levels were observed at the highest dose (30 mg/kg-day) in the P1 generation and T4 levels were significantly decreased in F1-generation also at a 30 mg/kg-day dose of perchlorate. The study is discussed in detail below.

Sprague-Dawley male and female rats (30/dose group) were treated with 0, 0.3, 3.0 and 30 mg/kg-day ammonium perchlorate in the drinking water (Argus Research Laboratories, Inc., 1999; as cited in U.S. EPA, 2002). One male and one female rat were allowed to cohabitate for a minimum of 14 days. Day one of lactation (LD1) was considered as the day of birth. Rats that did not deliver a litter were sacrificed on gestation day 25 for pregnancy status examination. All surviving parental generation (P1 or F0 generation) and pups not selected for further evaluation were sacrificed at PND21. The selected F1 generation pups were dosed during post-weaning, cohabitation, and lactation period.

Absolute thyroid weight was significantly increased in the parental (P1) generation males with a LOAEL of 3 mg/kg-day. In females, the LOAEL for this effect was 30 mg/kg-day. Thyroid weight was also increased significantly relative to brain and body weight. Benchmark dose analysis resulted in BMDL estimates of 0.11 mg/kg-day for colloid depletion and 2.44 mg/kg-day for hyperplasia (U.S. EPA 2002a).

Colloid depletion, hypertrophy and hyperplasia were also observed in the F1 (second parental generation, P2) generation at 3 and 30 mg/kg-day dose levels. Benchmark dose analysis gave 0.90, 0.15, and 0.0004 mg/kg-day as the BMDLs for colloid depletion, hypertrophy and hyperplasia respectively. In addition to the lesions (colloid depletion, hypertrophy and hyperplasia), two animals from the high dose group (30 mg/kg-day) in the F1 generation (second parental generation, P2) in the study had thyroid adenoma. These animals were dosed from conception to 19 weeks of age. Using historical control data and rigorous statistical analysis U.S.EPA (2002) determined this effect to be treatment-related. The BMDL (0.0004 mg/kg-day) associated with hyperplasia is the lowest in this group (Table 11) suggesting that the hyperplasia may be a lesion preceding the formation of tumors.

F1 weanling rats also exhibited thyroid colloid depletion, hypertrophy and hyperplasia. Benchmark dose analysis using the male and female data combined resulted in BMDL estimates of 0.8, 0.057, 0.66 mg/kg-day for colloid depletion, hypertrophy and hyperplasia respectively. In the second (F2) weanling generation, increases in colloid depletion, hypertrophy, and hyperplasia were observed at 3 and 30 mg/kg-day. The increase in hyperplasia was not remarkable. A BMDL of 0.32 mg/kg-day was determined only for hypertrophy. The BMDL across generations ranged from 0.11 to 0.9 mg/kg-day for colloid depletion, from 0.057 and 0.32 mg/kg-day for hypertrophy, and from 0.0004 to 2.44 mg/kg-day for hyperplasia.

The hormone analysis results demonstrated that T3 levels were increased in the P1 generation contrary to what would be expected, while the T4 levels were significantly decreased and TSH levels significantly increased as might be expected at the 30 mg/kg-day dose level. An unexpected increase in T3 was also found in the F1 generation (second parental generation, P2). Thyroid hyperplasia induced by iodine deficiency is associated with an altered pattern of thyroid hormonogenesis: the abnormal configuration of the poorly iodinated thyroglobulin in the thyroid colloid is accompanied by an increase in poorly iodinated compounds, monoiodotyrosine (MIT) and T3, and a decrease in diiodotyrosine (DIT) and T4. The increase of the MIT/DIT and T3/T4 ratios is closely related to the degree of iodine depletion of the gland (Ermans et al., 1963).

Table 11. Thyroid Histological Results of the 2-Generation Reproductive Study of Rats Treated with Various Doses of Perchlorate (Argus, 1999)

Generation	BMDL mg/kg-day (colloid depletion)	BMDL mg/kg-day (hypertrophy)	BMDL mg/kg-day (hyperplasia)	LOAEL mg/kg-day (thyroid adenoma)
P1	0.11	ND ^a	2.44	
F1 parental or P2	0.9	0.15	0.0004	30
F1 weanling	0.8	0.057	0.66	
F2 weanling	ND (poor model fit)	0.32	NOE ^b	

^aND = not determined

^bNOE = No observed effect: either no incidence of endpoint noted in animals tested or no notable differences between dosed and controls.

Similarly, the T3/T4 ratio in the serum is elevated in conditions of iodine deficiency probably because of thyroidal secretion of T4 and T3 in the proportion in which they exist within the gland and/or because of preferential secretion of T3 or increased peripheral conversion of T4 to T3. The shift to increased T3 secretion plays an important role in the adaptation to iodine deficiency because T3 possesses about 4 times the metabolic potency of T4 but requires only 75 % as much iodine for synthesis. Whether the unexpected increase observed in the F1 generation is due to any one of the above mechanisms is unknown.

Significant decreases in T4 were also detected in the F1 generation adult males at the high dose with an unexplained increase in the mid dose. TSH was significantly increased in adult males at the 30 mg/kg-day dose level. Similar results were reported for the F1 generation adult females.

7.2.6 Immunotoxicity

In rats and rabbits treated with 190 mg/kg-day perchlorate for three months, no immune-related effects were observed (Shigan 1963; as cited in CA EPA, 2002). The route of exposure was not specified.

A series of 14- and 90-day studies was conducted in female B6C3F1 mice (Keil et al., 1998; 1999; BRT-Burleson Research Technology Inc. 2000a,b,c) or CBA/J Hsd mice (BRT-Burleson Research Technology Inc. 2000a,b,c). The mouse was chosen for these studies because it is the typical experimental species for immunotoxicological studies.

Mice were exposed to levels of 0, 0.1, 1.0, 3.0, or 30 mg/kg-day perchlorate in drinking water in the Kiel et al. (1998; 1999) studies, while in the BRT-Burleson Research Technology Inc. studies mice were exposed to levels of 0.02, 0.06, 2 or 50 mg/kg-day. Immune function assays including innate, humoral, and cell-mediated were performed in perchlorate treated and control mice. Hematological investigations were also performed in these animals. No consistent alterations in many of the immune functions assays were observed. However, changes in lymphoproliferation, antibody response to sheep red blood cells (SRBCs), *ex vivo* phagocytosis, and local lymph node responses were observed due to perchlorate treatment.

Delayed type hypersensitivity (DTH) responses were measured by lymphoproliferation (LP) of splenic lymphocytes from *L. monocytogenes*-challenged mice incubated with soluble *Listeria* antigen (SLA). The results of this assay indicated an enhanced LP response in mice treated with 30 mg/kg-day in both the 14- and 90-day studies. The NOAEL in this assay was 3 mg/kg-day. Ant-IgM SRBC plaque-forming cell (PFC) assay was performed in control and perchlorate treated mice. This assay quantifies the number of plasma cells in the spleen which produce SRBC-specific IgM. The PFC assay showed no significant difference between controls and treated mice in the 14-day study. In the 90-day study, perchlorate increased the PFC response in the 2 and 50 mg/kg-day dose group. The NOAEL for this endpoint was 0.2 mg/kg-day. Based on the results of the assay it was postulated that perchlorate may enhance immune activity. Host resistance to infectious agents was measured by examining the phagocytic capacity of peritoneal macrophages in *ex vivo* and *in vivo* studies. Decreased *ex vivo* Phagocytosis of *L. monocytogenes* was observed at 3 and 30 mg/kg-day in the 14-day study. The NOAEL in this assay was 0.1 mg/kg-day. In the 90-day study, macrophage phagocytosis was decreased at all doses in all

groups. The LOAEL in this assay was 0.1 mg/kg-day. Decrease in phagocytic activity was not observed in an *in vivo* study.

Mice were exposed to perchlorate doses ranging from 0.02 to 50 mg/kg-day for 14 or 90 days. No differences were detected in all hematological parameters tested between perchlorate-treated and control animals. In humans treated with perchlorate, contact hypersensitivity was one of the adverse effects observed (Fawcett and Clarke, 1961; Hobson, 1961, Johnson and Moore; 1961; Southwell and Randal 1960, Barzilai, and Sheinfeld , 1966; Sunar, 1963). Because of this concern a local lymph node assay (LLNA) was performed in mice treated with perchlorate and challenged with the known contact sensitizer 2,4-dinitrochlorobenzene (DNCB). An ammonium perchlorate dose as low as 0.06 mg/kg-day enhanced the LLNA response.

7.2.7 Carcinogenic and Genotoxic Effects

Older data demonstrated that rats and mice chronically exposed to high concentrations of perchlorate (> 1000 mg/kg-day) developed thyroid tumors (Pajer and Kalisnik, 1991; Kessler and Kruskemper, 1966). More recent studies have investigated effects at lower level exposures and have also detected carcinogenic effects. These results are consistent with human epidemiological observations where increased rates of thyroid cancer have been reported in iodine deficient populations. Genotoxicity assays of perchlorate have yielded negative results, suggesting that this agent is likely to be acting through non-genotoxic mechanisms.

In a two-generation dose-response reproductive study conducted as part of the perchlorate testing strategy by Argus Research Laboratories, Inc. (1999), thyroid follicular cell adenomas were observed in the F1 generation male Sprague-Dawley rats (2/30) sacrificed as adults (P2-generation) at 19 weeks. Tumors occurred in animals exposed *in utero* and after birth to the highest perchlorate (30 mg/kg-day) dose only. Although the observed tumor rates were not statistically significant using standard tests, U.S.EPA (2002) applied Bayesian analysis using historical control data for the type of tumor and determined that the increase in follicular tumor incidence in these rats was statistically significant.

Ammonium perchlorate was negative in the Ames test using various strains of *Salmonella typhimurium* (ManTech Environmental Technology, 1998; Zeiger, 1998). Ammonium perchlorate was also negative in mouse lymphoma assays and in *in vivo* micronuclei assays in mice (ManTech Environmental Technology, 1998) and Sprague-Dawley rats Springborn Laboratories Inc., 1998). These data suggest that perchlorate may not be genotoxic.

8. DOSE-RESPONSE ANALYSIS

The first studies on perchlorate exposure in humans were performed in Graves' Disease patients. Graves' Disease is an immune disorder that is characterized by hyperthyroidism. But recently a number of animal and human clinical studies were conducted to fill data gaps. These clinical and animal studies are assessed below to determine an appropriate basis to derive an RfD for perchlorate.

8.1 CLINICAL STUDIES ON IODINE UPTAKE INHIBITION

Two clinical studies performed at low perchlorate doses (Greer et al., 2002, and Lawrence et al., 2001) were identified. These studies are discussed in detail in Section 7.1. In the study conducted by Lawrence et al. (2001), 0.14 mg/kg-day perchlorate significantly decreased radioactive iodide (^{123}I) uptake (RAIU) in healthy subjects with high iodine intake after 14 days of perchlorate administration in the drinking water.

Greer et al. (2002) administered perchlorate in drinking water at 0.007, 0.02, 0.1, or 0.5 mg/kg-day to 37 iodine sufficient healthy male and female volunteers for 14 days, and measured inhibition of radioactive iodide (^{123}I) (RAIU) uptake by the thyroid at different time points. Significant RAIU was observed in all groups except the lowest dose (0.007 mg/kg-day) (Table 4). The authors estimated the NOAEL to be 0.007 mg/kg-day. However, U.S.EPA (2002) statistically evaluated whether the 0.007 mg/kg-day treatment group had sufficient sample size to detect a difference of the magnitude observed in the other dose groups and found the power at the 0.007 mg/kg-day dose to be 0.1 compared to 0.95, 0.998, and 0.999 at 0.02, 0.1, and 0.5 mg/kg-day perchlorate doses respectively. U.S.EPA (2002) thus considered the lowest dose to be a minimally effective LOAEL. This report supports U.S.EPA's determination of 0.007 mg/kg-day as a minimally effective LOAEL.

8.2 HUMAN DEVELOPMENTAL STUDIES

Eight new ecological studies have been conducted since 1999. The U.S.EPA (2002; 2003) has extensively reviewed and critiqued these studies and concluded that these studies have significant scientific and technical limitations that prohibit their use as a basis for reference dose derivation for perchlorate that would be protective of public health. The detailed U.S.EPA assessment of these studies is found in U.S.EPA (2002; 2003) documents on perchlorate and readers are referred to these publications. ORS scientists and their joint science advisory committee have also concluded that these studies, due to a number of serious limitations, do not provide data useful to establishing an RfD for perchlorate.

One of the ecologic studies by Crump et al. (2002) was discussed briefly in Section 7.1.5 because of the wide attention it has received.

These authors studied 162 school children (mean age 7.5 years) and 9784 newborn babies in three Chilean cities that have different concentrations of perchlorate in the drinking water: Taltal (100 to 120 $\mu\text{g/L}$), Chanaral (5-7 $\mu\text{g/L}$) and Antofagasta (<5 $\mu\text{g/L}$). They reported that urine iodine concentrations were in the normal range in the school children. However, the mean urine iodine levels determined by Crump et al. (2002) in each of the cities are at least 30-fold higher than those reported by Soldin et al. (2003) for U.S. population from the analysis of the third National Health Nutrition Examination survey (NHANES III) data. Mean levels of TSH, T4, and T3 in the school children were similar among all three cities.

High prevalence of goiter was observed in Chanaral (26.5%), Taltal (23.3%) and Antofagasta (17%). Goiter prevalence was higher in Chanaral (5-7 µg/L) because this city was believed to have had iodine deficiency. The high background goiter prevalence in Antofagasta cannot be explained. Also, in this study, children with lifelong residence in Taltal were five times more likely to report a family history of thyroid disease compared to lifelong residents in Antofagasta (Table 12). The prevalence of goiter in these cities is much greater than estimates of goiter in U.S. children which range from 1 to 8 % (Xu et al., 1999; Hollingsworth et al., 1999, as cited in U.S. EPA, 2003). The prevalence in each is also greater than both the 4% reported for the thyroid-disease-free part of the general U.S. population or the 15% reported for the U.S. population self-reporting thyroid disease (including goiter) for the years 1988 to 1994 (Hollowell et al., 2002, as cited in U.S. EPA, 2003). If an area has a goiter rate >5% in children 6 to 12 years, it is classified as endemic with respect to goiter (U.S.EPA, 2003).

Table 12. Odds Ratio for Association Between Self-Reported Family History of Thyroid Disease¹ Among School Children and City of Residence² (Crump et al., 2000)

City	Concentration of Perchlorate in Drinking Water (µg/L)	School Children with less than life long residence (n=162)		School children with lifelong residence (n=127)	
		Odds Ratio	95% confidence Interval	Odds Ratio	95% Confidence Interval
Antofagasta	<5	1.0	-	1.00	-
Chanaral	5-7	0.89	0.25-3.19	1.04	0.21-5.09
Taltal	100-120	3.35	1.19-9.38	4.97	1.29-19.17

¹ Direct relative (parent, sibling, grandparent, great-grandparent, aunt, uncle, or cousin)

² Adjusted for age, sex and urinary iodine; excluded one child with autoimmune disease

Table adopted from (Crump et al., 2000)

Crump et al. (2000) also performed neonatal thyroid assessment. Linear regression comparison showed that mean log TSH values of newborns were significantly lower in Taltal than the other two cities. However, for the group of newborns sampled for day one and two, the mean and median values of TSH levels of Taltal were higher than those of Chanaral and Antafagosta. It is hard to reconcile these divergent results in TSH values and make any conclusion. Besides, the mean and median ages (day of life) at screening were different for the 3 cities raising the possibility of confounding of TSH values by age.

The Crump et al. (2000) study has a number of limitations, the most important being: a) the high background prevalence of goiter, family history of thyroid disease, and elevated urinary iodine excretion levels in the three cities; b) the lack of control for possible iodine ingestion differences across the three cities, and lack of data on iodine, nitrate, and iodate levels in the drinking water; c) the inadequacy of perchlorate exposure characterization; d) the lack of data on neurotoxicity, the sensitive and key endpoint in the sensitive subgroups, pregnant women and their fetuses; neonates and children; and, e) the lack of biomonitoring information documenting perchlorate exposure in the “high perchlorate” drinking water area.

Regarding a), the high prevalence of goiter and high urinary iodine levels in the study populations is troubling and is of concern given that the region is in an iodine excess state (U.S.

EPA, 2003). This study population and the location are inappropriate to study the effects of perchlorate. Moreover, the water used for drinking was treated with iodine (Mattie et al., 2003b). In the Crump et al. (2002) paper, passing mention is made of the fact that the nitrate deposits from which the high perchlorate water stems also contains high iodate levels. Neither iodate nor nitrate concentrations were measured in the drinking water samples collected to determine perchlorate levels. Since both of these compounds are known to interfere with iodide uptake (Eskandri, et al., 1997) into the thyroid gland they are potential confounders in the assessment of exposure to perchlorate and its toxicity. Regarding b), one would assume that those who are most at risk for perchlorate toxicity are those who obtain little iodine in their diet. Yet iodide levels in drinking water were not assessed in the 3 cities. Further, no attempt was made to compare dietary sources of iodine availability/supplementation across the 3 cities. The only marker of iodide status was urinary iodine concentration which was highly variable in school-age children from each city. Regarding d), thyroid gland function and structure were assessed in populations historically exposed to perchlorate for undetermined periods of time. Twenty-five contemporary community-wide water sources and the mean levels of perchlorate in these sources were estimated and were used as units of exposure for dose-response analysis. Individual exposure values were not determined in this study. It is also not known from the paper if replicates of each sample were taken for the measurement of perchlorate. Dose-response data obtained for perchlorate toxicity based on such incomplete exposure assessment could be misleading. Regarding e) it is now well established that mild to moderate iodine-deficiency in humans during pregnancy results in neuropsychological impairment in offspring having seemingly normal thyroid hormone levels. None of the ecologic studies reviewed in U.S.EPA (2002) including the Crump et al. (2000) study have investigated whether perchlorate exposure could be associated with neurological or behavioral problems in children. Vermiglio et al. (1990) studied children in iodine-deficient areas and observed defective visual perceptual integrative motor ability, but these children had T4 and T3 serum levels within the normal range. Regarding e), the lack of biomonitoring data is generally of concern in ecological studies but particularly so in this case where the perchlorate contamination of this Chilean city's drinking water is from a different source (mining releases) than what is experienced in the US – ammonium perchlorate from rocket propellants. This raises the question of chemical form and bioavailability of the Chilean perchlorate contamination relative to ammonium perchlorate. Salts of the smaller cations are very soluble, where as salts of the larger univalent cations (K^+ , Rb^+ , Cs^+) are less soluble (Wolf, 1998). How these various salts behave in the human body after ingestion is not known.

8.3 ANIMAL STUDIES

The results of the animal studies demonstrate that:

- (1) The target tissue for perchlorate toxicity is the thyroid gland;
- (2) The critical effect is inhibition of iodide uptake at the NIS (Yu et al., 2000);
- (3) Observed effects of uptake inhibition include:
 - (a) decreased thyroid hormone (T4 and T3) levels (Caldwell et al., 1995; Springborn Laboratories Inc, 1998; Research Laboratories Inc. 1998a, 1999, 2001);
 - (b) increased pituitary hormone (TSH) levels (Caldwell, 1995; Argus Research Laboratories, Inc., 1998a, 1999);

- (c) increased colloid depletion, hypertrophy and hyperplasia, that were the precursor lesions for tumor formation (Caldwell, 1995; Argus Research Laboratories, Inc., 1998a, 1999);
- (d) increased tumor incidence with decreased latency in the F1 generation rats that were exposed *in utero* and during development (Argus Research Laboratories, Inc., 1999); and
- (e) altered behavior (Argus Research Laboratories, Inc., 1998a; Bekkedal et al 2000) and brain morphometry (Argus Research Laboratories, Inc., 1998a; 2001) in rat pups that were exposed *in utero* and during lactation.

Tables 13 and 14 summarize studies and perchlorate dose levels associated with changes in thyroid hormone levels and thyroid structure.

Decreased thyroid hormone levels were observed at the 0.01 mg/kg-day dose level at different life stages, including the pregnant rat, the fetus and pups. TSH levels were also increased in adult rats at 0.01 mg/kg-day (Table 13).

Thyroid hypertrophy was observed at various life stages with doses of perchlorate ranging from 0.008 to 0.12 mg/kg-day. Brain morphometric alterations (especially changes in the size of the corpus callosum but also including the striatum and cerebellum) were observed at 0.01 mg/kg-day by PND21. Interestingly, decreased T4 levels were observed in the mothers of these pups.

Tumors were produced in the F1 generation at a young age in rats exposed *in utero* and after birth. However, the dose (30 mg/kg-day) that produced tumors in these rats was higher than the doses (0.009 to 0.33 mg/kg-day) that caused the precursor lesions, colloid depletion and hypertrophy (Table 14).

The major concern about perchlorate-induced iodine deficiency is the resultant alterations in thyroid and pituitary hormone levels which could result in neurotoxicity in human fetuses and children that are exposed to perchlorate *in utero* and during postnatal development. Various animal studies have confirmed that perchlorate treatment results in altered thyroid and pituitary hormone levels at various life stages. The LOAEL identified for thyroid and pituitary hormone disruption is 0.01 mg/kg-day (Table 13).

In a neurodevelopmental rat study conducted by Argus Research Laboratories, Inc. (2001), the dose (0.01 mg/kg-day) that was associated with thyroid and pituitary hormonal changes also caused changes in morphometry in various regions of the brain. These changes included an associated decrease in serum T4 levels (Table 13) and were seen in rats pups exposed *in utero* and during postnatal development. The dams in that study also had decreased T4 levels at GD21 which indicated that the pregnant animals might have been hypothyroid during pregnancy. However, the interpretations of structural changes observed in various brain regions of rat pups exposed *in utero* and during lactation have been challenged because of the way the brains were sliced for examination. The increase in the size of the corpus callosum in pups exposed *in utero* and after birth was observed in two different studies conducted by Argus Research Laboratories, Inc. (1998a and 2001). Size alterations were also observed in other brain regions, namely the

striatum and cerebellum. Because of the significance of brain developmental effects, MA DEP has chosen to consider these effects in the development of an appropriate RfD.

An increase in the size of the corpus callosum may represent a delay in the developing brain structure since this area is known to increase in size and then decrease later during development. Neurodevelopmental toxicity suggestive of delays was also demonstrated by effects on motor activity in both the Argus Research Laboratories, Inc. (1998a); and Bekkedal et al. (2000) studies (U.S.EPA, 2002).

Other studies using antithyroidal drugs such as methimazole or propylthiouracil have demonstrated that hypothyroidism could adversely affect the different brain regions including the corpus callosum. Cell adhesion molecules that are thought to play an important role in neuronal

Table 13. Perchlorate Dose Levels Associated with Changes in Thyroid and Pituitary Hormone Levels at Different Life Stages

Generation	T3 LOAELmg/kg-day	T4 LOAEL mg/kg-day	TSH LOAEL mg/kg-day	Brain morphometry (corpus callosum, striatum, cerebellum) LOAEL mg/kg-day	References
GD21 (Dams)		0.01 0.004 (BMDL)	0.01		Argus Research Laboratories, Inc., 2001
GD21 (PUPS)	0.01				Argus Research Laboratories, Inc., 2001
PND4, PND9	0.01				Argus Research Laboratories, Inc., 2001
PND21		0.01 (LOAEL) 2.86 x10 ⁻⁷ (BMDL)	0.01 (female)	0.01	Argus Research Laboratories, Inc., 2001
Adult rats		0.01	0.01		Springborn Laboratories Inc., 1998

differentiation and the establishment of connectivity during development were over-expressed after exposure to these drugs in the various regions of the developing brain including the corpus callosum (Alvarez-Dolado et al., 2001). Redistribution of the callosally-projecting cell somata was observed in the brains of hypothyroid rats (Gravel and Hawkes, 1990a). Another study demonstrated that the number of callosally projecting neurons seemed to be higher and structurally immature in hypothyroid rats than in normal controls (Gravel et al., 1990b). Cytoarchitectural changes in callosally projecting neurons and number of myelinated axons were observed in the progeny of hypothyroid rats (Berbel et al., 1993; Berbel et al., 1994; Barradas et al. 2001; Berbel et al., 2001).

Table 14. Perchlorate Dose Levels Associated with Thyroid Histopathology and Brain Morphometry Changes at Different Life Stages

Generation	Colloid depletion BMDL mg/kg-day	Hypertrophy BMDL mg/kg-day	Hyperplasia (BMDL)	Brain morphometry LOAEL mg/kg-day	Thyroid Tumor LOAEL mg/kg-day	Reference
PND4	0.33-0.009					Argus Research Laboratories, Inc., 1998; 2001
GD21		0.12 (male) 0.04 (female)				Argus Research Laboratories, Inc., 1998; 2001
F1 Weanling Pups	0.8	0.057	0.66		30	Argus Research Laboratories, Inc., 1999
F1 Parental or P2	0.9	0.15	0.0004			Argus Research Laboratories, Inc., 1999
Subchronic (14-day) Subchronic (19-day)	0.28 0.03	0.017 0.008				Springborn Laboratories Inc., 1998
PND21				0.01		Argus Research Laboratories, Inc., 2001

These studies collectively suggest that the corpus callosum was adversely affected by hypothyroidism induced by chemicals like perchlorate that target the thyroid gland. Although the mechanism by which thyroid hormone perturbation increases the size of the corpus callosum is not well understood at this time, this report considers the change in the size of the corpus callosum to be an adverse effect due to perchlorate exposure. The LOAEL for change in brain morphometry including the corpus callosum is 0.01 mg/kg-day perchlorate dose, and is included in the weight of evidence analysis used in this assessment.

8.4 IMMUNOTOXICITY AND HEMATOLOGICAL EFFECTS

In rats and rabbits treated with 190 mg/kg-day perchlorate for three months, no immune-related effects were observed (Shigan 1963; as cited in CA EPA, 2002). The route of exposure was not specified.

A series of 14- and 90-day studies was conducted in female B6C3F1 mice (Keil et al., 1998; 1999; BRT-Burleson Research Technology Inc..2000a,b,c) or CBA/J Hsd mic (BRT-Burleson Research Technology Inc.2000a,b,c) using perchlorate doses ranging between 0.1 and 50 mg/kg-day to study the immunotoxicity of this compound. The mouse was chosen for these studies because it is the typical experimental species for immunotoxicological studies.

Innate, humoral, and cell-mediated assays conducted in perchlorate treated and control mice indicated both immuno-enhancement and immunosuppression in perchlorate treated animals. Hematological investigations were also performed in these animals. No consistent alterations in many of the immune functions assays were detected. However, changes in lymphoproliferation,

antibody response to sheep red blood cells (SRBCs), *ex vivo* phagocytosis, and local lymph node responses were observed due to perchlorate treatment.

Delayed type hypersensitivity (DTH) response was enhanced in both 14- and 90-day studies, as measured by the lymphoproliferation (LP) assay in mice treated with 30 mg/kg-day perchlorate. The NOAEL in this assay was 3 mg/kg-day. Perchlorate also enhanced immune responses as measured by the Ant-IgM SRBC plaque-forming cell (PFC) assay. The PFC assay showed no significant difference between controls and treated mice in the 14-day study, but the response was enhanced in the 90-day study at 2 and 50 mg/kg-day dose group. The effect in this assay appears to be duration-dependent. The NOAEL for this endpoint was 0.2 mg/kg-day and might have been lower if the exposure had been chronic. Based on the results of this assay it is postulated that perchlorate may enhance immune activity. The immune system is a delicately balanced system, and immunoenhancement may lead to immune-mediated diseases such as hypersensitivity responses or autoimmune disease. Immunosuppression was established by decreases in *ex vivo* phagocytosis of *L. monocytogenes* at 3 and 30 mg/kg-day in the 14-day study. The NOAEL for this endpoint was 0.1 mg/kg-day. In the 90-day study, macrophage phagocytosis was decreased at all doses in all treated groups. The LOAEL in this assay was 0.1 mg/kg-day. It is interesting to note that with prolonged exposure diminished phagocytic responses were observed at lower doses, suggesting that chronic exposure may result in decreased immune activity at lower doses. A decrease in phagocytic activity was not observed in the *in vivo* studies lasting up to 90 days. Chronic studies are recommended to make an unequivocal conclusion on the immunosuppressive effect of perchlorate as evidenced by decreased phagocytic activity.

No differences were detected in all hematological parameters tested between perchlorate-treated and control animals in this series of mouse studies. In humans treated with perchlorate contact hypersensitivity was one of the adverse effects observed (Fawcett and Clarke, 1961; Hobson, 1961, Johnson and Moore; 1961; Southwell and Randal 1960, Barzilai, and Sheinfeld, 1966; Sunar, 1963). Because of this concern a local lymph node assay (LLNA) was performed in mice treated with perchlorate and challenged with the known contact sensitizer 2,4-dinitrochlorobenzene (DNCB). Ammonium perchlorate doses as low as 0.06 mg/kg-day enhanced the LLNA response. It is not clear why mice were not treated with perchlorate and then challenged with perchlorate to see if perchlorate is a direct dermal sensitizer.

There are no experimental studies in humans regarding immunotoxicity. In an *in vitro* study, using human T and B cells, perchlorate was shown to have an immunosuppressive potential at concentrations that were not cytotoxic (Weetman et al., 1984). similar comparative *in vitro* studies using mouse T and B cells were not identified in the literature. However, the results of the studies conducted in mice indicated enhancement of some immune responses.

The major concerns about the immunotoxicity studies conducted in mice are the duration of exposure, and the relevance of the observed results in mice to humans. Some the positive results discussed previously suggest that observed effects occur either after longer exposure periods or after longer exposure duration with effects occurring at lower doses. The effect of chronic perchlorate exposure on the immune system may not be addressed in such short-term assays. The

only treatment-related effect that occurred at low enough dose was dermal contact hypersensitivity. The LOAEL identified for this effect was 0.06 mg/kg-day.

No hematological effects were observed in either the 14- or 90-day studies using oral perchlorate treatment. This is in contrast to the human data where humans exposed to oral doses of perchlorate show various hematological effects. It is interesting to note that 9-14 mg/kg-day perchlorate doses produced agranulocytosis, lymphadenopathy, and skin rash in humans while rats and rabbits treated with 190 mg/kg-day perchlorate did not show any immune-type responses.

Thus, the available data on the immunotoxicity and hematological effects of perchlorate provide a limited basis upon which to assess potential long-term, low dose risks of perchlorate. Moreover, rodents appear to be less sensitive to the immunotoxicity of perchlorate than humans. Future investigations should consider more relevant species like primates to study the immunotoxic and hematologic effects of perchlorate.

8.5 CARCINOGENIC EFFECTS

The available animal studies have demonstrated that when TSH is stimulated due to reduced thyroid hormone (T4 and T3) levels, hypertrophy, hyperplasia and eventually thyroid neoplasms result. Perchlorate was not found to be genotoxic in various *in vivo* and *in vitro* assays (ManTech Environmental Technology Laboratories Inc., 1998; as cited in U.S.EPA, 2002; Zeiger, 1998; Inc), further supporting the proposed mechanism of action for perchlorate that perturbation of the hypothalamus-pituitary-thyroid axis may lead to tumor formation.

The historical data have demonstrated that rats and mice chronically exposed to high concentrations of perchlorate (> 1000 mg/kg-day) produced thyroid tumors (Pajer and Kalisnik, 1991; Kessler and Kruskemper, 1996).

In two-generation reproductive studies conducted as part of the perchlorate testing strategy by Argus Research Laboratories, Inc. (1999), thyroid follicular cell adenomas were observed in the F1 generation sacrificed as adult (P2-generation) male Sprague-Dawley rats (2/30) at 19 weeks. Tumors occurred in animals exposed *in utero* and after birth to the highest perchlorate dose (30 mg/kg-day) only. Although the observed tumor rates were not statistically significant using standard tests, U.S.EPA (2002) applied Bayesian analysis using historical control data for the type of tumor and determined that there were significant increases in follicular tumor incidence in these rats.

Regarding rat carcinogenicity data, U.S.EPA (2002) concluded that in spite of potential qualitative similarities between rodents and humans, there is evidence that humans may not be as sensitive quantitatively to thyroid-pituitary disruption-induced thyroid cancer as rodents. This situation is suggested to be due to differences between thyroid hormone protein carriers in the two species. Humans have a high affinity binding protein (thyroxin-binding globulin), which binds T4 avidly and T3 to a lesser extent. This protein is missing in rodents and rabbits. The absence of this high affinity protein in these lower animals was hypothesized to cause high turnover rates of thyroid hormones because of increased metabolism of more freely available

thyroid hormones. As a result, the rodent thyroid gland is chronically stimulated by elevated TSH levels compensating for the increased turnover rate of thyroid hormones.

In contrast to the above hypothesis, ORS believes that there are no data to support that rats are in fact more sensitive than humans. In mammals, thyroid hormones (T4 and T3) are associated with control of metabolism. Rodents have higher metabolic rates than humans which are related to their body size and lifespan. In studies conducted in the later half of the 1970s, it was shown that metabolic activation and deactivation of polycyclic aromatic hydrocarbons (PAHs) (Schwartz, 1975; Schwartz and Moore, 1977; 1979), and the DNA binding capacity of reactive metabolites (Schwartz and Moore 1977) were inversely correlated to species longevity. The capabilities of DNA repair mechanisms across a spectrum of species were mathematical functions of lifespan, and it was postulated that interspecies capabilities would be about the same if life span was factored in. There are no studies showing how thyroid hormone turnover rate is correlated to species longevity and size, and without such studies it would be premature to conclude that rodents are more susceptible to perchlorate-induced thyroid tumor formation than humans based on thyroid turnover rate alone.

9. TOXICITY VALUE DERIVATIONS

9.1 CARCINOGENICITY

U.S.EPA has prepared a science policy guidance document regarding thyroid carcinogenesis (U.S.EPA, 1998), and recommends a margin of exposure dose-response analysis procedure based on nonlinearity of effects when thyroid-pituitary disruption is judged to be the sole mode of action of the observed thyroid and related pituitary tumors.

Chemicals leading to tumor formation due to thyroid-pituitary disruption should demonstrate: increase in thyroid cellular growth, thyroid and pituitary hormone changes, site of action, dose correlation, reversibility of effects following cessation of treatment, and lesion progression (U.S. EPA, 1998). Based on the reviewed data, perchlorate seems to have fulfilled the above requirements and RfDs for perchlorate were determined based on precursor lesions to tumor formation (colloid depletion and hypertrophy).

Various genotoxicity assays have also demonstrated that perchlorate is not likely to be mutagenic. This was an important consideration in the choice of a nonlinear dose-response approach for deriving a protective dose level for carcinogenic effects. Using adult male rat dosimetric adjustments, U.S.EPA (2002) identified human equivalent exposures (HEE) of 0.45 and 0.02 mg/kg-day for colloid depletion and hypertrophy respectively. Using the nonlinear approach and applying an uncertainty of 100 to the HEE (for human variability, duration, and database deficiencies) an RfD in the range of 0.005 to 0.0002 mg/kg-day was derived for colloid depletion and hypertrophy respectively. Applying a larger uncertainty factor of 300 resulted in a range of 0.002 to 0.00007 mg/kg-day for colloid depletion and hypertrophy, respectively. U.S.EPA (2002) uses this analysis to conclude that the derivation based on tumor outcome data supports the mode-of-action concept and indicates that the proposed RfD based on thyroid and pituitary hormone changes and brain morphometry alterations would be protective of both neurodevelopmental and neoplastic sequelae. ORS concurs with EPA's analysis.

9.2 NONCANCER TOXICITY (CALCULATION OF RfD)

ORS has used a weight of the evidence approach by critically evaluating the available animal and human data on perchlorate toxicity to calculate an RfD for this compound. In selecting data for setting the RfD, the concordance of effect levels observed for several endpoints, including perchlorate-induced iodide uptake inhibition in humans and animals, thyroidal and pituitary hormone alterations and changes in brain and thyroid morphometry in animals as well as the perchlorate proposed mode of action provide a strong basis for establishing an RfD. Key data evaluated in the RfD derivation process are discussed below.

ORS has identified two key data sets for use in the RfD derivation process for perchlorate: (1) the neurodevelopmental animal study by Argus Research Laboratories, Inc. (2001) and (2) the human iodide uptake inhibition study by Greer et al. (2002).

9.2.1 Animal Studies

The results of the various studies discussed previously demonstrated that the key target tissue for perchlorate toxicity is the thyroid gland, and the critical events are alterations in thyroid and pituitary hormone levels, and changes in thyroid and brain morphometry.

The LOAELs identified for hormone perturbation, in studies at various life stages were at or near 0.01 mg/kg-day. Significant changes in T4 and TSH levels were observed in GD21 dams and TSH levels in PND9 dams at a perchlorate dose of 0.01 mg/kg-day, and this dose was identified as a LOAEL (Argus Research Laboratories, Inc., 2001). When the benchmark dose model was fit to the T4 dose-response data from the GD21 dams (US EPA, 2002), the BMDL for this endpoint was calculated to be 0.004 mg/kg-day. T3 levels were also decreased in GD21 fetus, PND4 and PND9 pups, and the LOAEL for this endpoint was also 0.01 mg/kg-day. BMDL estimates were extremely sensitive for changes in T4 at PND21 in males with a BMDL value of 2.86×10^{-7} mg/kg-day respectively (Table 15). It is significant to note that T4 was depressed in the mother on GD21 also. It is the decrease in T4 in the mother, especially, during the early pregnancy that is associated with neurodevelopmental deficit in offspring in humans.

The data on thyroid histopathologic changes were analyzed using the benchmark dose model (US EPA, 2002). A benchmark response level of 10% was used for each of the indices (colloid depletion, hypertrophy and hyperplasia) to determine the lower 95% confidence limit on the dose (BMDL). BMDLs that caused thyroid colloid depletion at different life stages ranged from 0.009 to 0.33 mg/kg-day (Springborn Laboratories Inc. 1998; Argus Research Laboratories, Inc., 1998a, 2001). The lowest doses associated with increased thyroid hypertrophy ranged from 0.008 – 0.32 mg/kg-day (Springborn Laboratories, Inc. 1998; Argus Research Laboratories, Inc., 1998a, 1999). And the lowest dose for thyroid hyperplasia was 0.0004 mg/kg-day (Argus Research Laboratories, Inc., 1999). The lowest values in the ranges identified for thyroid and pituitary hormone changes, and thyroid and brain structural alterations are presented in Table 15. Consistent with the proposed mode of action of perchlorate, changes in thyroid hormone levels were associated with altered brain morphometry. The neurodevelopmental study conducted by Argus Research Laboratories, Inc. (2001) demonstrated that perchlorate exposure *in utero* and

during early development caused: (1) thyroid and pituitary hormone changes in dams and pups; and (2) alterations in morphometry at various regions of the brain (anterior and posterior corpus callosum, striatum and cerebellum) in pups. The LOAEL identified for the various changes in brain morphometry was 0.01 mg/kg-day (Table 8, Table 15). Questions were raised during the EPA public comment period on their 2002 draft perchlorate document regarding the way the brains were sliced and measured to determine the sizes of the brain regions, especially the corpus callosum. U.S. EPA has recently addressed the alleged measurement bias between controls and treated animals in the corpus callosum in their Response to Comments Document (EPA, 2003) and continues to maintain that the data indicate that perchlorate caused statistically significant increase in the size of the corpus callosum. An increase in the size of the striatum was also confirmed in male rat brains in the reanalysis of the brain sections. The results observed in various regions of the brain have been included in the weight of evidence evaluation. DEP is following developments in this area.

Changes in motor activity were also observed in pups exposed in *utero* and postnatally and the NOAEL identified for this effect was 1mg/kg-day (Argus Research Laboratories, Inc., 1998; Bekkedal et al. 2000) (Table 15), a level substantially higher than that associated with alterations in the other endpoints discussed above.

Table 15 Summary of Endpoints and Associated Lowest Values of LOAELs and BMDLs Used in the Weight of Evidence Approach to Deriving an RfD for Perchlorate

Species	Endpoints Evaluated at Different Life-Stages	LOAEL (mg/kg-day)	BMDL (mg/kg-day)	References
Rat	T4 Levels	0.01 GD21 dams	¹ 2.86 x 10 ⁻⁷ (PND21) ¹ 0.004 (GD21 dam)	Argus, 2001; Springborn, 1998
	T3 Levels	0.01 GD21 fetus, PND4 and PND9 pups		Argus, 2001; Springborn, 1998
	TSH Levels	0.01 GD21 dams, PND21dams		Argus, 2001; Springborn, 1998
	Thyroid Colloid Depletion		² 0.008 ³ 0.009	Argus, 2000 Argus, 1998a
	Thyroid Hypertrophy		⁴ 0.008	Springborn, 1998
	Thyroid Hyperplasia		0.0004 (P2 Generation)	Argus, 1999
	Brain Morphometry Changes (corpus callosum, striatum, cerebellum)	⁵ 0.01 (PND21 pups)		Argus, 2001,
	Behavioral effects	1 (NOAEL)		Argus, 1998; Bekkedal et al., 2000
Human	Radio Iodide Uptake Inhibition (RIUI)	⁶ 0.007	0.002 (U.S EPA 2003)	Greer et al., 2002

¹See Table 10

²See Section 7.2.5

³See Section 7.2.4

⁴See Table 7

⁶See Table 8

⁷See Table 4

Choice of Point of Departure and RfD Derivation

Since change in thyroid status and brain morphometry were observed in replicated studies, the corresponding LOAEL (0.01 mg/kg-day ammonium perchlorate, 0.0085 mg/kg-day perchlorate anion) at which these effects were observed was selected as the point of departure for the derivation of an RfD. This value was chosen in preference to the BMDL values derived by U.S.EPA because it was consistently observed in multiple studies, for multiple endpoints, and was generally supported by the BMDL values for thyroid histopathology. The BMDL values were considered but not selected as a basis for calculating a toxicity value for perchlorate because of their great variability and questions about the applicability of the BMD approach to the data.

The derivation of an RfD from the LOAEL is represented as:

$$\text{RfD} = \frac{\text{LOAEL}_{\text{HED}}(\text{mg/kg-day})}{\text{UF}} \quad (\text{Equation 1})$$

Where:

- RfD = Reference dose, mg/kg-day
 LOAEL_(HED) = Lowest observed adverse effect level, human equivalent dose in mg/kg-day
 UF = Uncertainty factor

The application of composite uncertainty factors of either 300 or 1000 to the LOAEL can be supported, resulting in RfD values ranging from 9×10^{-6} to 3×10^{-5} mg/kg-day. The derivation of these values is presented and discussed below.

$$\text{RfD} = \frac{0.0085 \text{ mg/kg-day}}{300} \approx 3 \times 10^{-5} \text{ mg/kg-day (higher range value)}$$

UF: 300

- 10 = human variability;
- 10 = LOAEL to NOAEL; and,
- 3 = animal to human

$$\text{RfD} = \frac{0.0085 \text{ mg/kg-day}}{1000} \approx 9 \times 10^{-6} \text{ mg/kg-day (lower range value)}$$

UF: 1000

- 10 = human variability;
- 10 = LOAEL to NOAEL;
- 3 = animal to human; and,
- 3 = database deficiency

The justifications for the use of each factor are presented in the following paragraphs.

(1) 10- Human Variability

The human variability uncertainty factor is applied to account for variations in susceptibility within the human population (interhuman variability) and the possibility (given a lack of relevant data) that the database available is not representative of the dose/exposure-response relationship in the subgroups of the human population that are most sensitive to the health hazards of the chemical being assessed (U.S. EPA, 2002b). Although the animal studies were conducted in the most sensitive subgroups, the pregnant animal and the fetus and the developing pups, there is great potential for differences in toxicokinetics of iodide and perchlorate uptake, distribution and excretion and toxicodynamic responses to perchlorate exposure as well as interactions of iodine deficiency with other processes, even among the sensitive subgroups studied. Moreover, the rats used in the toxicity studies are bred for homogeneity while the human population is heterogeneous.

Dourson and Stara (1983) analyzed acute lethality data in animals for a large number of chemicals and concluded that use of a 10-fold factor to account for intraspecies variability in lieu of chemical-specific data was appropriate. Various authors who have evaluated the human variability uncertainty factor (as summarized by Dourson et al. (1996) have concluded that the 10-fold default factor appears to be protective when starting from a median response – by inference a NOAEL is assumed to be from an average group of humans (US EPA, 2002b). Korsovskii (1976) reported that a 6-fold difference in sensitivity to the effects of nitrates in children, and a general 3- to 5-fold difference in sensitivity between children and adults. The intraspecies variability factor determined from the Korsovskii (1976) data ranged between 18 and 30.

(2) 3- Animal to Human Extrapolation

The interspecies uncertainty factor is applied to account for the extrapolation of laboratory animal data to humans, and is generally presumed to include both toxicokinetic and toxicodynamic aspects. Seldom are there data available to inform about toxicodynamic differences. One-half of the default 10-fold interspecies uncertainty factor (i.e., $10^{0.5}$) is assumed to account for such differences. Unless data support the conclusion that the test species is more or equally as susceptible to the pollutant as are humans, and in the absence of any other specific toxicokinetic or toxicodynamic data, a default factor of 10 is applied (U.S EPA, 2002b).

In this assessment, the recommended default factor of 10 was reduced to 3 (i.e., approximately $10^{0.5}$), because of the availability of data on toxicokinetic differences between humans and test animals and between different life stages of both humans and test animals. There were no data on toxicodynamic differences.

Extrapolation based on perchlorate distribution (toxicokinetic differences) and iodide uptake inhibition by perchlorate at lower doses was characterized by PBPK modeling (U.S.EPA 2002). PBPK model-predicted doses associated with various degrees of iodide inhibition (Mattie et al., 2003a) suggested that humans were more sensitive than rats for perchlorate-induced iodide

uptake inhibition by at least a factor of 2 at low doses (Table 16). No such comparisons could be made between animals and humans regarding perchlorate's effect on thyroid and pituitary hormone alterations and brain development but there is no reason to believe that humans would be less sensitive. Moreover, no assessment exists on toxicodynamic differences in responses to perchlorate between species and between different life-stages, especially regarding thyroid hormone interaction with its nuclear receptors at various sites in the complex human and rat brain, and the ensuing effects on gene expression. The relative sensitivities of the laboratory animal assays used to characterize the types of neurodevelopmental deficits related to maternal hypothyroidism in humans are unknown. Based on the data, an uncertainty factor of 3 is not an overly conservative estimate to address species differences.

Table 16. Relative Doses Resulting in 5, 10, or 20 % Iodide Uptake Inhibition (Rat VS Human)

	Normal Adult	Pregnant Female	Fetus (M & F)	Lactating Female	Neonate (M&F)
RAT	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
5% inhibition	0.03 (male rat)	0.050	0.0120	0.130	0.080
10% inhibition	0.050 (male rat)	0.090	0.0230	0.230	0.150
20% inhibition	0.079 (male rat)	0.135	0.0430	0.400	0.260
HUMAN	mg/kg-d	mg/kg-d	mg/kg-d	mg/kg-d	mg/kg-d
5% inhibition	0.014 (M&F)	0.023	0.006	0.061	0.037
10% inhibition	0.029 (M&F)	0.052	0.013	0.133	0.087
20% inhibition	0.064 (M&F)	0.109	0.035	0.324	0.211

Values in the lower shaded columns represent the proportional differences between PBPK-derived values for the male rat and the human applied PBPK-derived predictions for the pregnant, fetal, lactating and neonatal rats. Table modified and adopted from Mattie et al. (2003b).

(3) 10- LOAEL to NOAEL Extrapolation

A standard uncertainty factor of 10 is typically applied when a NOAEL is not available. The size of the LOAEL-to NOAEL uncertainty factor may be altered, depending on the magnitude and the response at the LOAEL (U.S.EPA, 2002b). In this case, a factor of 10 is justifiable given that effects on multiple endpoints were observed including altered brain structure and impacts on thyroid hormone levels.

In the study used to derive the RfD, perchlorate administration decreased T4 levels in both GD21 dams and PND21 pups at the identified LOAEL of 0.01 mg/kg-day. Benchmark dose estimates were very sensitive for changes in T4 at PND21 with a BMDL of 2.86×10^{-7} (Table 13). In the GD21 dams, the BMDL for change in T4 levels was 0.004 mg/kg-day. These data suggest that the LOAEL for hormone disruption may be significantly lower than 0.01 mg/kg/day. It is therefore appropriate to apply a full uncertainty factor of 10 to extrapolate from LOAEL to NOAEL, in order to be health protective.

(4) 1 or 3- Database Deficiency

Although considerable uncertainty exists regarding perchlorate toxicity, the overall data-base on this compound is quite extensive. Thus, ORS has chosen to use UFs of either 1 or 3 to address database deficiencies. Some of the data base deficiencies are discussed below.

a) Information on the chronic effects from long-term exposure of perchlorate in general (even in neurotoxicity) is lacking. This is especially of concern because of the increase in thyroid tumors in F1-generation rats at 19 weeks. These animals were exposed to perchlorate from conception to 19 weeks of age. The reduced latency period of these tumors suggests that perchlorate exposure may have induced *in utero* disruption of thyroid function and structure that progressed to tumor at a young age. It is not known how these animals would be affected if exposure were continued over their lifetime. Progressive effects of exposure duration on hormone levels and thyroid morphometry were observed in rats (Spring born, 1998) and healthy humans (Brabant et al., 1992) at short-duration of exposures.

b) Exposure duration and other limited information on perchlorate immunotoxicity is also a concern. For example, in the studies conducted to investigate the immunotoxicity of perchlorate in mice, the plaque-forming cell (PFC) assay described previously in Section 7.2.6, showed no significant difference between controls and treated mice in the 14-day study while in the 90 day study, perchlorate increased the PFC response in the 2 and 50 mg/kg-day dose group. Decreased *ex vivo* phagocytosis of *L. monocytogenes* was observed at 3 and 30 mg/kg-day in the 14-day study. The NOAEL in this assay was 0.1 mg/kg-day. In the 90-day study, however, macrophage phagocytosis was decreased at all doses in all groups. The LOAEL in this assay was 0.1 mg/kg-day. These data suggest that immunotoxicity studies conducted over short exposure periods may not represent immune-related effects that may occur upon chronic exposure to perchlorate. The observed effects may also occur at lower doses upon chronic perchlorate exposure.

As described earlier, in humans clinically treated with perchlorate, dermal hypersensitivity was one of the adverse effects observed. Because of this concern a local lymph node assay (LLNA) was performed in mice treated with perchlorate and challenged with the known skin contact sensitizer 2,4-dinitrochlorobenzene (DNCB). An ammonium perchlorate dose as low as 0.06 mg/kg-day enhanced the LLNA response. It is not clear why mice were not treated with perchlorate and then challenged with perchlorate to see if this compound is a direct dermal sensitizer. Moreover, the dose associated with such an effect upon chronic exposure is unknown. Also, the results of the 14-day and 90-day studies demonstrated that perchlorate enhanced the LLNA activity at the highest dose in the 14-day study but suppressed it in the 90-day study. The mechanism of these duration-related divergent results is not understood.

Weetman et al. (1984) investigated the effect of perchlorate on human T and B cell responses to a mitogen *in vitro*, and found that perchlorate had a significant immunosuppressive activity at pharmacologically relevant concentrations that was not due to simple cytotoxicity. The observed effects could be due to the antithyroid or direct effect of perchlorate (U.S. EPA, 2002). Although the mechanism by which perchlorate exerts its effect on human T and B cells is not known, the results of the study suggest that human immune cells may be sensitive to perchlorate treatment and warrant further studies using relevant species like primates.

Comparative evaluation of the mouse hematological data and the historical perchlorate data suggest that mice, rats and rabbits may not be good models to study the hematologic effects of perchlorate. Graves' Disease patients treated with perchlorate doses ranging from 6 to 14 mg/kg-day developed skin rashes and various hematologic effects. Some of the hematological effects were fatal (Fawcett and Clarke, 1961; Hobson, 1961, Johnson and Moore; 1961; Southwell and Randal 1960, Barzilai, and Sheinfeld, 1966; Sunar, 1963). No hematologic effects were observed in mice treated with perchlorate dose up to 50 mg/kg-day for up to 90 days.

The hematologic and immunotoxic effects that occur in humans are not unique to perchlorate treatment. Other antithyroid drugs such as methimazole and propylthiouracil are also known to cause similar effects (Meyer-Gessner, 1989; Werner et al., 1989; Bartalena et al., 1996, Tavintharan et al., 1997). There are studies indicating that thionamide drugs, such as methimazole, that are used to treat Graves' Disease patients have direct effects on the immune system as demonstrated in *in vitro* and *in vivo* studies in animals (Davies, et al., 1984) and in clinical studies in humans (Lechpammer et al., 2002). Unlike perchlorate, rodents appear to respond to the immunotoxic effects of these chemicals. For perchlorate, however, rodents may not be a sensitive species. Until well-designed studies on perchlorate in sensitive species are completed, a direct effect of perchlorate on the immune and hematologic system cannot be ruled out.

While the above data gaps justify a database uncertainty factor, an alternative view is that the factor of 3 may not be warranted since the animal data set is so robust.

9.2.2 Human Radio Iodide Uptake Inhibition (RAIUI) Study

An initial step in the mechanism of perchlorate toxicity is inhibition of iodide uptake by the thyroid gland. Short-term clinical studies conducted in healthy adult individuals have demonstrated that exposure to fairly low concentrations of perchlorate inhibited iodide uptake in the exposed people (Greer et al., 2002; Lawrence et al., 2001).

Only the Greer et al. (2002) investigation used multiple doses and demonstrated dose-response. The study examined RAIUI in perchlorate-dosed healthy volunteers. Although this study is limited because of sample size, duration of exposure, and use of healthy, iodine-sufficient people

who do not represent the most sensitive subgroups (pregnant women, fetuses, children and hypothyroid individuals) the information from this study was included in the weight of the evidence evaluation and a range of RfD values was derived using appropriate uncertainty factors. The minimally effective LOAEL for iodide uptake inhibition identified in this study was 0.007 mg/kg-day (Table 15).

9.2.2.1 LOAEL-Based RfD Derivation

Uncertainty factors ranging between 100 and 300 were applied to the LOAEL to derive RfD values of 2.3×10^{-5} and 7×10^{-5} . The rationale for the use of a different uncertainty factors is discussed below.

$$\text{RfD} = \frac{\text{LOAEL}_{(\text{mg/kg-day})}}{\text{UF}} \quad (\text{Equation 1})$$

Where:

RfD = Reference dose, mg/kg-d
 LOAEL_(mg/kg-day) = Lowest observed adverse effect level in mg/kg-day
 UF = Uncertainty factor

$$\text{a) RfD} = \frac{0.007 \text{ mg/kg-day}}{100} = 7 \times 10^{-5} \text{ mg/kg-day (higher value of range)}$$

UF: 100

- 10 = human variability;
- 3 = uncertainty in the NOAEL; and,
- 3 = database deficiency

$$\text{b) RfD} = \frac{0.007 \text{ mg/kg-day}}{300} = 2.3 \times 10^{-5} \text{ mg/kg-day (higher value of range)}$$

UF: 300

- 10 = human variability;
- 3 = uncertainty in NOAEL; and,
- 10 = database deficiency

The rationale for the various uncertainty factors applied is as follows:

(1) 3 - Uncertainty in the Greer et al. Identified NOAEL

As described previously, a default uncertainty factor of 10 is typically applied when a NOAEL is not available. The size of the LOAEL-to NOAEL uncertainty factor may be altered, depending on the magnitude and the response at the LOAEL (U.S.EPA, 2002b).

The authors of the Greer et al. (2002) study identified the 0.007 mg/kg-day dose of perchlorate as the NOAEL for inhibition of iodide uptake by the thyroid. This NOAEL value is very uncertain. While the study authors used larger number of subjects in all the higher dose groups (10 subjects per dose group), they included 30% (7 subjects) fewer subjects in the lowest dose group, and the results in this low dose group were highly variable. The small sample size and great variability in measured RAIUI values place this NOAEL value in question. U.S.EPA (2002) evaluated whether the 0.007 mg/kg-day group had a sufficient sample size to detect a difference of the magnitude observed at the other doses tested. They found the statistical power at the 0.007 mg/kg-day dose to be 0.1 compared to 0.95, 0.998, and 0.999 at the 0.02, 0.1, and 0.5 mg/kg-day perchlorate dose levels, respectively. Thus, the results observed at 0.007 mg/kg-day cannot be concluded to be a NOAEL.

U.S EPA (US EPA, 2003) used the benchmark dose (BMD) model and estimated a BMDL value corresponding to a five percent reduction of the mean thyroidal iodide uptake of 0.002 mg/kg-day. The U.S EPA considered the BMDL to be equivalent to NOAEL. The BMDL value of 0.002 mg/kg-day is equivalent to the LOAEL from the Greer study, adjusted by a factor of 3 for LOAEL to NOAEL extrapolation ($0.007/3 = 0.002$ - rounded), justifying an uncertainty factor of 3 for this parameter when using the LOAEL identified from the Greer et al. data.

(2) 10 - Human Variability

As described previously, the human variability uncertainty factor is applied to account for variations in susceptibility within the human population (interhuman variability) and the possibility (given a lack of relevant data) that the database available is not representative of the dose/exposure-response relationship in the subgroups of the human population that are most sensitive to the health hazards of the chemical being assessed (U.S. EPA, 2002b).

In this assessment, a factor of 10 for human variability accounts for differences in inter-individual sensitivity within neonates, children or adult population and differences in sensitivity between adults and other life-stages. The human short-term study was conducted in a limited number healthy young volunteers who were iodine-sufficient. The study groups do not represent the sensitive subpopulations (pregnant woman, fetuses, children, hypothyroid individuals, and people with thyroid disease). As described previously, there are studies (Korsovskii, 1976; Dourson and Stara 1983) that support an uncertainty factor of 10 for human variability.

Some have suggested that iodide uptake inhibition is comparable between fetuses, adults and neonates because of the structural similarity of the NIS protein, and that fetuses, neonates and children should not be any more sensitive than adults. Although the protein structure of the NIS protein is similar across life-stages, the functional maturity of the protein and its microenvironment regarding iodine uptake into the thyroid is not well understood. For example, in 1948, Wolff and Chaikoff (as cited in Eng et al., 1999) reported that the organic binding of iodide in the thyroid was decreased when plasma

iodide levels were elevated (acute Wolff-Chaikoff effect), and that adaptation or “escape” from the acute effect occurred in approximately 2 days, in the presence of continued high plasma iodide transport into the thyroid, lowering the intrathyroidal iodine content below a critical inhibitory threshold and allowing organification of iodide to resume. The “escape” from the acute Wolff-Chaikoff effect is caused by the down regulation of the NIS protein, with a resultant decrease in iodide transport into the thyroid despite high levels of iodide in the serum (Eng et al., 1999). In newborns and fetuses, unlike the adult thyroid, the escape from the inhibitory effect of large doses of iodide is not achieved and hormone synthesis remains inhibited causing clinical or subclinical hypothyroidism. This phenomenon suggests that the autoregulation mechanism involving the NIS is not well developed in newborns and fetuses (Woeber, 1991; Markou et al., 2001). Moreover, there is another protein, pendrin, that may be involved in the transport of iodine from the apical membrane of the thyrocyte into the colloid space (Scott et al., 2000). The structural and functional similarity of this protein among different life-stages and how it is affected by perchlorate is not known.

In addition, differences in sensitivity to inhibition of the NIS and resulting alterations in thyroid function are likely. The Greer et al. (2002) study estimates that there should be sufficient hormone stored in the thyroid gland to last for several months (referring to healthy adults). The case is quite different for the late gestation fetus or neonate. Vulsma et al. (1989) estimated that the neonatal thyroid gland contains thyroid hormone equivalent to only a single day’s secretion. This estimate was revised by Van den Hove et al. (1999) who empirically measured intrathyroidal stores of thyroid hormones in human fetuses and neonates and found that the amount of hormones stored in the colloid is less than that required for a single day. Thus the concentration of perchlorate sufficient to reduce thyroidal iodide uptake in a fetus or neonate may be sufficient to produce a significant decrease in circulated levels of thyroid hormones.

(3) 3 or 10 - Database Deficiency.

A database deficiency UF of either 3 or 10 is supported in this case, as discussed below.

The duration of exposure in the Greer study was only 14 days, during which iodide uptake inhibition of the thyroid was measured. Long-term effects due to iodide uptake inhibition, iodide discharge, thyroid accumulation of perchlorate, thyroid hormone perturbation, or direct chronic effects of perchlorate in various other organs cannot be determined from this study. It has been argued that perchlorate is readily eliminated and does not accumulate to cause chronic effects.

Whether perchlorate is translocated into the thyroid cells or bound to them is a matter of debate. However, the clearance of perchlorate exhibits a biphasic pattern with the $T_{1/2}$ of the majority (>90%) of perchlorate within a couple of hours and the $T_{1/2}$ the remainder 70-80 hours (Von Burg, 1995; as cited in U.S. EPA, 2002). This pattern is consistent with a multi (at least 2) compartment model, with accumulation and slow clearance from at least one compartment. Part of this slow clearing compartment is

likely to be the thyroid. Accumulation of perchlorate over time in the thyroid could well lead to duration-dependent effects.

Experimental studies in humans and animals suggest that prolonged exposure to perchlorate causes progressive effects at lower doses in humans (Brabant et al., 1992; Brabant et al., 1994 as cited in U.S. EPA, 2002) and rats (Springborn Laboratories Inc., 1998).

Brabant et al. (1992) studied five healthy male volunteers pretreated with 200 µg/day iodine for four weeks before perchlorate exposure. Iodine exposure was discontinued, and the volunteers were given 13 mg/kg-day of potassium perchlorate for another four weeks. Perchlorate treatment did not change serum total T3 or T4 levels or thyroid gland volume. However, serum free T4, TSH, and intrathyroidal iodine concentration levels were significantly diminished and thyroglobulin serum levels were almost doubled, indicating the stress of the treatment on the thyroid function. In a follow-up study, Brabant et al. (1994) repeated the earlier studies with perchlorate treatment lasting longer than 4 weeks. As a result of the longer treatment, thyroid volumes increased in all subjects although TSH levels did not increase. The increase in thyroid volume without an increase in TSH levels is not consistent with the hypothalamic-pituitary-thyroid feedback mechanism, suggesting that the mechanism of perchlorate's action on the hypothalamus-pituitary-thyroid feedback mechanism is not well understood.

In rats treated with perchlorate for either 14 or 90 days (Springborn Laboratories Inc., 1998), thyroid hormone levels after 90 days of treatment were decreased at lower dose levels than was observed after 14 days of exposure. Progressive changes in thyroid structure were also observed with duration of exposure in this study. Fatal aplastic anemia (Trotte, 1962; Krevans et al., 1962; Gjerdal, 1963; Barzilai and Scheinfeld, 1966) was observed in Graves' Disease patients after 2-6 months exposure suggesting that duration was a contributing factor for the observed effects in these patients (Wolf, 1998).

Characteristics unique to children should be considered when applying data from normal adults to the potential developmental consequences. In animals, perchlorate was found in higher concentrations in breast milk of treated animals than in the serum (Yu, 2000; as cited in CA EPA, 2002). It is possible that perchlorate is concentrated in human breast milk since the NIS protein is expressed in this tissue. However, there are no studies to determine the relationship between perchlorate intake in nursing mothers and the dose of perchlorate presented to her infant.

Perchlorate not only inhibits iodide uptake but also promotes rapid and nearly complete discharge of iodide from the thyroid gland. A single dose of 1.4 mg/kg-day perchlorate caused complete discharge while a single dose of 0.14 mg/kg-day caused 50% release of stored iodide from the thyroid gland of Graves' Disease patients. Potassium perchlorate doses as low as 0.04 mg/kg-day caused detectable but incomplete release of iodide from the thyroid of these patients (Stanbury and

Wyngaarden, 1952). The mechanism by which perchlorate accomplishes the discharge of stored thyroidal iodide and how this process affects thyrocytes upon short- and long-term exposure is not well understood.

Perchlorate is a potent thyroid tumor promoter in mice (Hiasa et al., 1998). It is not known how and at what dose level perchlorate will affect humans with an already initiated thyroid cancer.

Other relevant database gaps related to immunotoxicity were discussed previously under database deficiency in the RfD derivation section using the animal data.

The information discussed above clearly supports the need for an UF for database deficiency. While a factor of 10 is justifiable, because of the significant amount of data available, an UF of 3 could be argued to be sufficient. Thus, UFs of 10 and 3 were applied in the RfD derivation.

9.2.2.2. Benchmark Dose-Based RfD Derivation

An alternative method to the NOAEL/LOAEL approach for deriving an RfD using the Greer et al. (2002) data is the benchmark dose (BMD) approach. The BMD approach fits a statistically-based model curve to the dose-response data being evaluated. The equation for the curve is used to predict the dose associated with a chosen low, but statistically reliable, response level. Ten percent (dichotomous data) or 5% (continuous data) response levels have most often been chosen as the default point of departure since these response levels are at or near the limit of sensitivity in some bioassays (U. S.EPA, 2000).

The Greer et al. (2002) data appear to adequately fit the Hill model. The Hill model is the one most often used for evaluation of continuous outcome measures such as RAIU (U.S. EPA, 2003).

The General form of the Hill model for a response (RAIU inhibition) is

$$X = \beta_0 - v \times \text{Dose}^n / (k^n + \text{Dose}^n) + \varepsilon$$

Where: β_0 is the intercept, expected to be 1 as X is a ratio relative to base;
 v is the “velocity”, with $v > 0$ and $\beta_0 \geq v$ expected;
 k is scale parameter; and
 n is the exponent with $n \geq 1$ expected

The U.S.EPA identified two outliers from the low dose group in the Greer study. The 24-hour sample set from the study without the two outliers on exposure day 14 was considered as the most appropriate data set for deriving a chronic risk estimate. The U.S. EPA determined the BMDL estimate for a benchmark response of 5% to be 0.002 mg/kg-day and applied a total uncertainty factor of 100 (10 for database deficiency and a 10 for human variability) to this BMDL to calculate an RfD of 2×10^{-5} mg/kg-day. This value is similar to the RfD derived by ORS using the same data with the NOAEL/LOAEL approach and application of a total uncertainty factor of 300 (3 for uncertainty in the NOAEL, 10 for human variability, and 10 for database deficiency).

10. SUMMARY

The human studies reviewed indicate that the most sensitive subgroups for iodine deficiency are pregnant women and their fetuses, children, hypothyroid individuals and those with thyroid disease. The initial target site for perchlorate toxicity is the thyroid gland.

After reviewing all the available human and animal data on the toxicity of perchlorate, ORS has used the weight of evidence approach to determine an RfD for perchlorate. The data assessed included: (1) a battery of animal studies addressing thyroid toxicity, developmental neurotoxicity, developmental/reproductive toxicity, mutagenicity/genotoxicity, carcinogenicity, and immunotoxicity; and, (2) a dose-response human study on radioiodide uptake inhibition. A range of uncertainty factors was applied to the animal and human LOAELs from these studies to derive a range RfDs. The RfD derivation process is summarized in Figure 6.

In animals, the lowest dose tested (0.01 mg/kg-day) caused thyroid and pituitary hormone changes with accompanying brain and thyroid morphometric alterations. Uncertainty factors of 300 and 1000 (Figure 6) were applied to this LOAEL to derive RfD values bounding a range between 8.5×10^{-6} and 3×10^{-5} mg/kg-day.

In humans, perchlorate inhibited radioiodide uptake by the thyroid gland. A range of RfD values were derived from the human radioiodide uptake inhibition study using the NOAEL/LOAEL approach. The LOAEL for this effect was 0.007 mg/kg-day and the uncertainty factors applied to this LOAEL were 100 and 300, resulting in RfD values of 2.3×10^{-5} and 7×10^{-5} mg/kg-day.

The RfDs based on the animal and human data overlap between 2.3×10^{-5} and 3×10^{-5} (Figure 6), a very narrow range. **Since MA DEP as a rule develops single RfD numbers and not a range of values, a point estimate of 3×10^{-5} mg/kg-day was selected as the RfD. This value is chosen because of the more robust animal database and is supported by the weight of the evidence.**

11. CONCLUSIONS AND RECOMMENDATIONS

Based on the analysis of the animal and human data, this report concludes that thyroidal radioiodide uptake inhibition, thyroid and pituitary hormone alterations and brain morphometric effects from low level perchlorate exposure are an appropriate basis from which to derive an RfD. **This report recommends a perchlorate RfD of 3×10^{-5} mg/kg-day for MA DEP use** which is protective of public health, including those members of the population at most risk from perchlorate exposure.

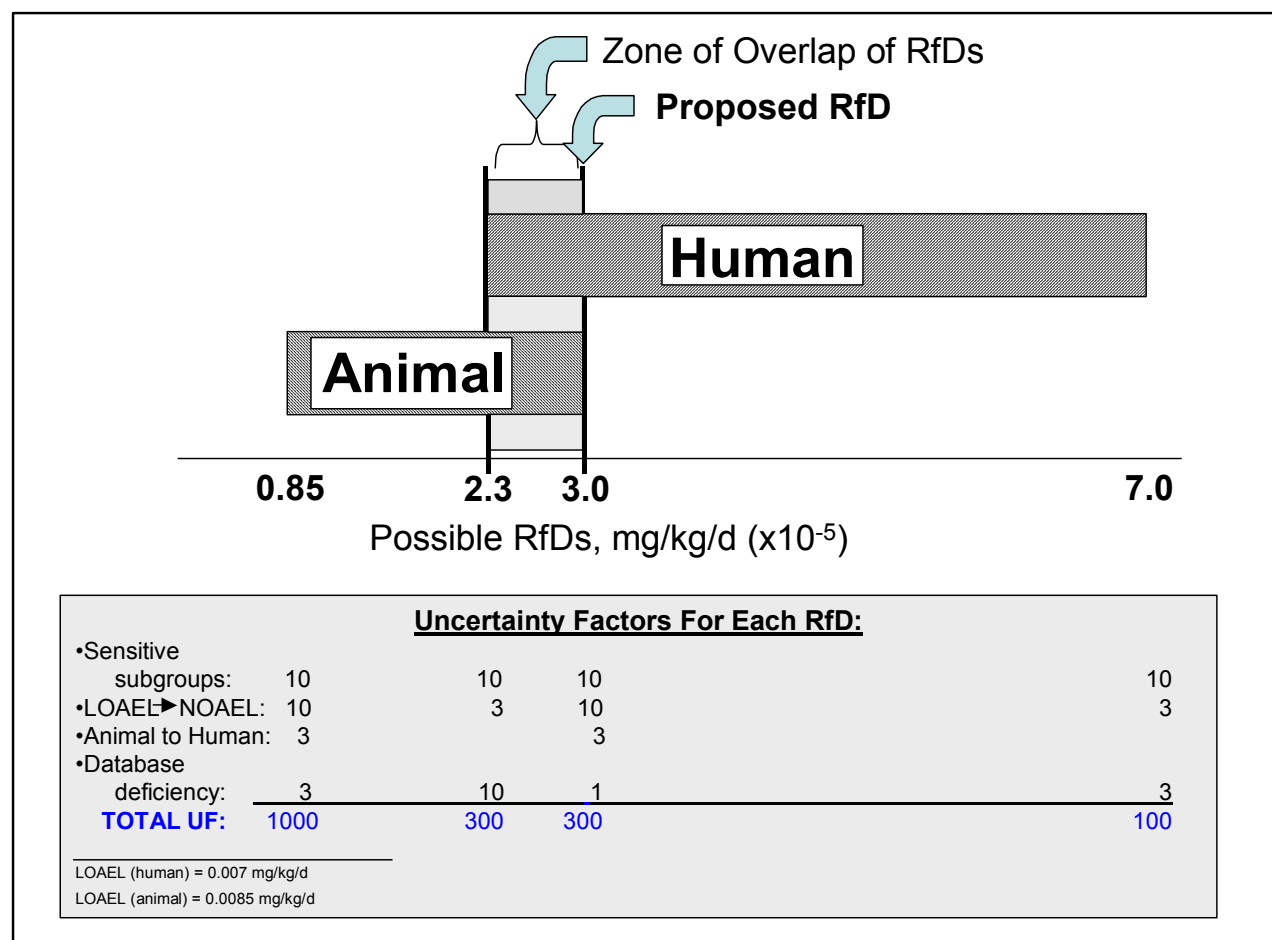


Figure 6. Summary of the RfD Derivation Process and RfD Ranges

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APPENDIX A

In October of 2003, the U.S. EPA released responses to peer review comments on its draft perchlorate toxicological review and risk characterization of 2002 (U.S. EPA, 2003). The objective of the 2002 draft report was to present the results of a review of the toxicological literature for perchlorate and identify an oral reference dose for perchlorate. This document followed two previous external peer reviews and public comment processes. The most recent document details in depth the U.S. EPA's evaluation and responses to the comments of its peer reviewers and contains numerous clarifications of conclusions and additional interpretation of the data used. MADEP has reviewed this document and finds that it provides considerable additional support for the proposed MA RfD for perchlorate. A number of the key conclusions in the U.S. EPA response to comments that have a bearing on MA DEP's assessment are briefly summarized below. In general, the U.S. EPA has not changed its position on the toxicity of perchlorate. Although several revisions to its upcoming final perchlorate document are proposed by the U.S. EPA, the numerical value of the RfD for perchlorate derived in its 2002 document has not changed and remains at 3×10^{-5} mg/kg-day.

The latest U.S. EPA document includes more thorough integration of the variety of biological response data into a coherent picture of the mode of action of perchlorate within humans and animal models. Key additions consist of additional analysis of the rat brain morphometry, more detailed mathematical analysis of the dose-response characteristics of the Greer et al. (2002) human exposure study, and consideration of various endpoints as points of departure for derivation of the reference dose for perchlorate.

In response to the considerable criticism of earlier rat brain sectioning of the corpus callosum conducted as part of one of the key studies (Argus, 2001), U.S. EPA supported the generation and analysis of new sections of original tissue blocks of the control PND21 male rat brains and re-measured the existing brain sections of perchlorate treated PND21 male rats for comparison. The results from this work supported the original evaluation, indicating significant effects of perchlorate treatment on regional brain growth characteristics. This analysis identified a LOAEL of 0.01 mg/kg-day, which was consistent with that determined from the original data analysis presented in the U.S. EPA (2002) draft document. Based on this new information, DEP has concluded that it is appropriate to include the corpus callosum sections with the striatum and cerebellum in its weight of evidence evaluation, while noting that the data on hormonal effects alone support DEP's proposed RfD.

U.S. EPA's more detailed analysis of the Greer et al.'s (2000, 2002) data on humans exposed to perchlorate included evaluation of study design characteristics on the biological response monitored and better characterization of the nature of the dose-response relationship using statistical techniques. The objective of this more intensive assessment was to help better inform decisions on the choice of a point of departure for derivation of a final RfD. The U.S. EPA document noted that reviewers raised a number of issues with the Greer et al. study, including: its lack of information on subjects (health status; ethnicity; weight; etc.); the limited number of subjects included in the study; the short duration of the study; and the lack of data on potentially susceptible individuals. These issues introduce considerable uncertainty in the use of this data for deriving an acceptable exposure value for the particularly susceptible segments of the population, and argue against sole reliance on this study for this purpose. Important conclusions from U.S. EPA's response to comments were that the 2-week exposure duration employed in the

study was insufficient as a basis for estimating chronic exposure effects, supporting use of an uncertainty factor for exposure duration in the derivation of an RfD. The mathematical characterization of the dose-response relationship, accounting for the small number of subjects in the study permitted the identification of an estimated NOAEL of 0.002 mg/kg-day perchlorate for iodide uptake inhibition in euthyroid adult humans

Reviewer comments regarding the use of other human data derived from epidemiological studies were also addressed. In summary, U.S. EPA concluded that the human epidemiological data on perchlorate are limited and insufficient to determine a health protective RfD value. This conclusion is in agreement with DEP and DEP's advisory committee positions on this issue.

U.S. EPA has provided a synthesis of the toxicological studies for a variety of animal response endpoints to perchlorate exposure and illustrated that the level of concern for the initiation of observable perchlorate effects centers around 0.01 mg/kg-day. The variety of effects which show initial sensitivity at this level (LOAELs) include serum hormone changes in the adult, pregnant, fetal and neonatal animals, thyroid weight changes in pups, and brain morphometric changes in pups. These clustered LOAELs can be compared with the newly calculated NOAEL of 0.002 mg/kg-d from the Greer et al (2002) study. Since LOAELs are traditionally divided by an uncertainty factor of 10 to represent extrapolation from a LOAEL to a NOAEL in the process of deriving an RfD, the NOAEL derived from the LOAEL of 0.01 mg/kg-day would be 0.001: quite similar to the human-based value. U.S. EPA's synthesis agrees with MA DEP's approach in its evaluation where two key studies with LOAELs of 0.01 mg/kg-day were highlighted.

In summary, the U.S. EPA response to comments document provides additional support, including new data, for MA DEP's proposed RfD for perchlorate.

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